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School of Fisheries, WH-10
University of Washington
Seattle, WA 98195

**ANNOTATED BIBLIOGRAPHY OF BIOASSAYS
RELATED TO SEDIMENT TOXICITY TESTING
IN WASHINGTON STATE**

PAUL A. DINNEL

FINAL REPORT

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SEATTLE DISTRICT,
U.S. ARMY CORPS OF ENGINEERS
SEATTLE, WASHINGTON 98124

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<p>This bibliography is directed toward the support of sediment bioassays being conducted in the Puget Sound region. However, it also includes information on water column bioassays which may contain methods or results pertinent to sediment assays. This is often the case since many sediment bioassays are adaptations of earlier water column assays (e.g., embryo/larval assays, Microtox).</p> <p>The bibliography addresses seven basic areas:</p> <p><u>Chapter</u></p> <ol style="list-style-type: none"> 1. Methods—Protocols—Reviews, 2. Amphipod bioassays 3. Embryo/larval bioassays 4. Polychaete bioassays 5. Microtox (bacterial luminescence) bioassays 6. Geoduck bioassays 7. Multiple testing protocols 															
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INTRODUCTION

This annotated bibliography was compiled during the author's part-time, temporary assignment to the Seattle District, U. S. Army Corps of Engineers (COE) under the provisions of an Intergovernmental-Personnel Act (IPA) agreement. My primary task was to assist with the planning, coordination and review of sediment bioassays being conducted for the Puget Sound Dredge Disposal Analysis (PSDDA) program.

During the planning and review of bioassay projects with the COE, it became apparent that consistency in the use of bioassay methodologies between projects and contract laboratories needed some refinement. In addition, interpretation of the results of sediment bioassays was often problematical due to the interactive effects of multiple variables (e.g., exposure times, test temperatures, salinities, pHs, sediment grain sizes, etc.) and the lack of a historical perspective on past bioassays conducted in the Puget Sound region or in other geographical areas that may contain information germane to the local situation. Thus, this bibliography evolved as I reviewed past bioassay studies in an effort to provide a sense of continuity and conformity with past work.

This bibliography is directed toward the support of sediment bioassays being conducted in the Puget Sound region. However, it also includes information on water column bioassays which may contain methods or results pertinent to sediment assays. This is often the case since many sediment bioassays are adaptations of earlier water column assays (e.g., embryo/larval assays, Microtox).

The bibliography addresses seven basic areas:

Chapter

1. Methods—Protocols—Reviews
2. Amphipod bioassays
3. Embryo/larval bioassays
4. Polychaete bioassays
5. Microtox (bacterial luminescence) bioassays
6. Geoduck bioassays
7. Multiple testing protocols

The first chapter generally includes information on the conduct of bioassays in general. Chapters 2-6 contain entries specific to each of those bioassays. Amphipod bioassay citations focus primarily on tests conducted with *Rhepoxynius abronius*; embryo/larval citations deal primarily with oyster, mussel and echinoderm species; polychaete assays with *Neanthes arenaceodentata* testing; and multiple testing citations with studies that have used two or more assays concurrently (as is specified for PSDDA sediment testing). The entries in most chapters are subdivided into the following specific areas:

- Methodologies
- Sediment testing
- Water column testing
- Reviews and/or miscellaneous information

All entries are listed in the typical alphabetical/chronological style used in most "Literature Cited" sections of scientific reports or publications. For most of the annotated bibliographical entries, the following information is provided:

- A full literature citation
- A brief summary of the study

- Detailed information on the methods and test conditions
- A detailed summary of the results and conclusions, often with data summary tables

This bibliography is far from exhaustive. However, it does cover a substantial amount of material, especially as related to amphipod, embryo/larval and *Neanthes* testing, and field studies conducted in the Puget Sound region. For each entry there exists a copy of the original article in a special set of files located in the Environmental Resources Section of the Seattle District, U. S. Army Corps of Engineers, Federal Center South, Seattle.

CHAPTER 1. METHODS—PROTOCOLS—REVIEWS

GENERAL

Anderson, B. S., J. W. Hunt, M. Martin, S. L. Turpen and F. H. Palmer. 1988. Marine bioassay project, third report. Protocol development: Reference toxicant and initial complex effluent testing. Report No. 88-7WQ, Division of Water Quality, California State Water Resources Control Board, CA. 154 pp.

This is the third in a series of reports covering the development of marine bioassays for use by the State of California for monitoring effluent and marine water quality. This report covers the refinement of three new tests and provides protocols for conducting these tests. The merits of various inorganic reference toxicants (all metals) are also discussed with the final selection of $ZnSO_4$ as the preferred reference toxicant. Interlaboratory tests with zinc and test effluents were also conducted.

Bioassay test summaries:

Red abalone, *Haliotis rufescens*: Short-term test = 48-hour early larval development test for shell abnormality. Long-term (calibration) test = 9-day metamorphosis success test.

Giant kelp, *Macrocystis pyrifera*: Short-term test = 48-hour germination and growth of settled zoospores. Long-term test = 15 day sporophyte production.

Opposum shrimp, *Holmesimysis (Acanthomysis) costata*: Short-term test = 48 or 96-hour acute lethality test using 3-day old juveniles. Long-term test = growth and survival endpoints following 21-day exposures.

Results of testing (No Observed Effect Concentrations (NOEC's)):

For Zinc (μ g/liter):

Species	Test 1	Test 2	Test 3
Abalone, 48-hour 9-day	40 19	41	37
Mysid, 48-hour 96-hour 21-day	182 100 45	175	320
Kelp, 48-hour germination 48-hour growth 16-day	2033 <1090 1071	5495 <589 <589	1732 <553 <553

Interlaboratory Zinc Tests (µg/liter zinc):

Species	MBP Lab	SCCWRP Lab
Abalone, 48-hours	37	18
Mysid, 96-hours	89	66
Kelp, 48-hour germination 48-hour growth	957 <538	----- <559

Complex Effluent Tests (% effluent):

Species	Primar Effluent	Secondary Effluent
Abalone, 48-hour		10
Mysid, 96-hour	1.0	32
Kelp, 48-hour germination 48-hour growth	0.56 -----	18 32

Other tests also given preliminary consideration:

- 1) Mussel, *Mytilus* sp., larval assay.
- 2) Squid, *Loligo opalescens*, juvenile assay.
- 3) Fish (white sea bass, kelp bass, northern anchovy) eggs and juveniles.
- 4) Mysid, epibenthic, *Metamysidopsis elongatus*.

Four metals were considered for use as a reference toxicant. The final rating for preference was Zn > Cd > Cu > Ag.

EPA (U. S. Environmental Protection Agency). 1978. Bioassay procedures for the Ocean Disposal Permit Program. EPA-600/9-78-10. 121 pp.

This is an update of the 1976 EPA Manual, EPA-600/9-76/010.

This manual gives detailed guidelines (not "standard" EPA methods) for evaluating materials for disposal in the ocean. This manual does not directly address sediment testing, *per se*.

Testing and culture instructions for the following types of bioassays are given:

Phytoplankton and zooplankton
Copepods
Mysids
Grass shrimp

Oyster (adult shell deposition)
Fish and macroinvertebrates
Fish brain acetylcholinesterase

FAO (Food and Agricultural Organization of the United Nations). 1977. Manual of methods in aquatic environment research. Part 4 - Bases for selecting biological tests to evaluate marine pollution. FAO Fisheries Tech. Paper No. 164, FAO, Rome. 31 pp.

This is a general bioassay guide for beginners, primarily for bioevaluation of marine pollutants. Discusses pollution in general, purposes and types of bioassays and factors affecting test organism selection.

Kobayashi, N. 1984. Marine ecotoxicological testing with echinoderms. Vol. 1, pp. 341-405
In: Ecotoxicological Testing for the Marine Environment, G. Persoone, E. Jaspers and C. Claus, editors. State University of Ghent and Institute of Marine Scientific Res., Bredene, Belgium.

This is a review paper dealing with the use of echinoderms (primarily sea urchins) for ecotoxicological testing, both field and laboratory. Topics covered include: Species, test methodologies for gamete, embryo and larval bioassays and a discussion of other physiological tests. This review also gives a wealth of data on the effect levels of various pollutants and natural waters and the differences in sensitivity between selected species.

Reish, D. J. and P. S. Oshida. 1987. Short-term static bioassays. Part 10 *In: Manual of Methods in Aquatic Environment Research*. FAO Fisheries Tech. Paper #247, Food and Agricultural Organization of the United Nations, Rome. 62 pp.

This is a very general bioassay manual for conducting static bioassays with both freshwater and marine organisms. The introduction discusses the purpose of bioassays and describes general categories of bioassay types. General bioassay procedures and laboratory requirements are then given for various groups of bioassay organisms, and includes a discussion of data analyses. Subsequent sections discuss bioassay procedures with selected groups of organisms (including phytoplankton, zooplankton, annelids, crustaceans, aquatic insects, molluscs (adults only), echinoderm larvae and fish), and bioassay procedures with selected toxicants (metals, petrochemicals, pesticides, contaminated sediments and liquid effluents).

Stephan, C. E. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. EPA-660/3-75-009, Office of Research and Development, U. S. Environmental Protection Agency, Corvallis, OR. 60 pp.

This is an acute bioassay manual designed by an EPA-sponsored committee on methods for toxicity tests with aquatic organisms. Discusses acute toxicity testing in general and four basic techniques: Static, Static Renewal, Recirculation, and flow-through systems. Gives detailed discussions on: Equipment, exposure systems, dilution waters (including recipes for reconstituted fresh and seawaters), test organisms, test procedures, data collection, and data analyses.

SEDIMENTS

Chapman, P. M. 1987. Marine sediment toxicity tests. Pp. 391-402 *In: Chemical and Biological Characterization of Sludges, Sediments, Dredge Spoils, and Drilling Muds*, J. J. Lichtenberg, F. A. Winter, C. I. Weber and L. Fradkin, eds. ASTM STP 976. Am. Soc. Testing and Materials, Philadelphia, PA.

This paper reviews use of bioassays for sediment toxicity assessments. It covers acute lethal, sublethal, cytotoxic/genotoxic and microbial activity assays. It discusses the following topics: Sediment collection, homogenizing and storage; QA/QC; test organisms; controls; water quality; and exposure routes. Recommends that amphipod (*Rhepoxynius abronius*) assays be used as a benchmark bioassay for comparing other techniques. It also discusses the regulatory use of toxicity tests.

COE/EPA (U. S. Army Corps of Engineers/U. S. Environmental Protection Agency). 1984. Guidance for performing tests on dredged material to be disposed of in ocean waters. Manual prepared by the U. S. Army Corps of Engineers, Regulatory Branch, New York and the U. S. Environmental Protection Agency, Region II, New York. 15 pp. + appendix.

The methods specified in this regional manual follow the general guidelines of the EPA/COE "Implementation Manual." Liquid-phase bioassays not required. Reference sediments must be collected near the disposal area. Any control mortalities >10% invalidate a test.

Suspended-phase tests are to be conducted with *Acartia tonsa*, *Menidia menidia*, and *Mysodopsis bahia* in duplicate, 10 organisms/replicate, static 96-hr exposures.

Solid-phase tests are to be conducted in a flow-through system for 10 days with 20 organisms/replicate. Test organisms = *Palaemonetes pugio*, *Mercenaria mercenaria*, and *Nereis virens*. Depths of control and reference sediments in aquaria = 45 mm and in the test aquaria = 30 mm reference sediment + 15 mm test sediment.

Bioaccumulation tests (with same organisms) are also discussed.

EPA/COE (U.S. Environmental Protection Agency/U.S. Army Corps of Engineers). 1978. Ecological evaluation of dredged material into ocean waters; Implementation Manual for Section 103 of Public Law 92-532 (Marine Pollution, Research, and Sanctuaries Act of 1972), July 1977. EPA/COE Technical Commission on Criteria for Dredged and Fill Material. Environmental Effects Laboratory, U. S. Army Waterways Experiment Station, Vicksburg, MS. 24 pp + appendices.

This article gives definitions and procedures for preparing liquid, solid and suspended particulate phase elutriates of sediments and for conducting appropriate bioassays of each phase.

Liquid Phase = Centrifuged and 0.45 μm -filtered supernatant remaining after 1 hr undisturbed settling of mixture resulting from vigorous 30-min agitation of a 1:4 ratio of sediment with site water. Discusses species and procedures for this and the other phase assays.

Suspended Particulate Phase = The supernatant, prior to centrifugation and filtration, obtained by the liquid-phase procedure.

Solid Phase = All material settling to the bottom within 1 hr in the liquid-phase procedure. Also, in practice, bottom sediments of *in situ* density may be considered to represent the solid phase.

Liquid-phase assays should include 3 species consisting of one plankton stage, one crustacean or mollusc, and one fish. Suspended-phase assays should include zooplankton, a crustacean or mollusc, and a fish. Solid-phase assays should include 3 species consisting of a filter-feeder, a deposit-feeder, and one burrowing species. Solid-phase tests should include a crustacean, an infaunal bivalve, and an infaunal polychaete.

Note: Planktonic stages, (e.g., larvae) are not recommended for use with solid-phase tests in this manual.

Long, E. R. 1983. A multidisciplinary approach to assessing pollution in coastal waters. Pp. 163-177 *In: Proc. Third Symposium on Coastal and Ocean Management*, ASCE/San Diego, CA, June 1-4, 1983.

This is a review/discussion paper dealing with the chemical and biological assessments of pollution, especially in marine sediments. Regarding sediment toxicity, general results of various testing programs are presented including amphipod bioassays, oligochaete respiration and anaphase aberrations in trout cells.

Olsen, L. A. 1984. Effects of contaminated sediment on fish and wildlife: Review and annotated bibliography. Final Rpt. for the U. S. Fish and Wildlife Service, Washington, D. C. FWS/OBS-82/66. 103 pp.

This review provides brief overviews of availability, bioaccumulation and "summary" of the effects of sediments contaminated with heavy metals, petroleum hydrocarbons, synthetic organic compounds and radionuclides. It also provides an annotated bibliography of several hundred sediment-related studies/publications.

Pierson, K. B., L. D. Tornberg, J. W. Nichols, G. C. McDowell and R. E. Nakatani. 1984. A bioassay protocol for evaluation of potential chemical toxicity from disposal of dredged sediments in Washington State. Final Rpt., Contract No. DACW56-79-C-0095, for Seattle District, U. S. Army Corps of Engineers by the Fisheries Research Institute, University of Washington, Seattle, WA. 116 pp.

This is a general review and guidance manual for conducting sediment bioassays, especially as related to the Pacific Northwest. It provides a discussion on the legal requirements and frameworks regarding the use of bioassays and strongly-worded critiques of the "Interim Bioassay Guidelines", the "WES Manual" and the "TerEco Manual."

This report discusses various guidelines for bioassay methodology including static vs. continuous-flow, species selection, sensitivity, availability, etc. It covers potentially useful bioassay organisms including amphipods, clams, various fish species, copepods, Dungeness crab, mussels, oyster, sea urchins, worms, shrimp and mysids. It also discusses experimental variables and design, statistical analyses and data interpretation, and bioassay facilities.

Reish, D. J. and J. A. Lemay. 1988. Bioassay manual for dredged sediments; U.S. Army Corps of Engineers, Los Angeles District. Research Rpt. for the Los Angeles District, U. S. Army Corps of Engineers by Reish Marine Studies, Inc., Los Alamitos, CA. 37 pp. + appendices.

This is a bioassay manual for testing sediment toxicity in Southern California and follows the general guidelines and specifications of the EPA/COE (1977) "Implementation Manual."

Various species are reviewed for use in solid, suspended particulate, and bioaccumulation assays. It gives a list of 15 species suitable for use and defines which type of exposure is appropriate. Echinoderm embryo assays are specified for liquid and suspended particulate-phase assays only. Gives various appendices covering grain size, chemical and statistical analyses of sediments and bioassays. Also gives collecting and culture details for 13 species. This manual also provides 96-hr LC50 data for the toxicity of 10 toxicants (As, Cd, Cr, Cu, Hg, Pb, Zn, DDT, PCB, and oil) to a list of 20 marine animals (Table 3 in the manual). Also, bioconcentration factors are given for 9 toxicants to 15 species of animals (Table 4).

Striplin, P. L. 1988. Puget Sound Ambient Monitoring Program marine sediment implementation plan. Final Rpt. for Washington Department of Ecology and the Puget Sound Water Quality Authority by Washington Department of Ecology, Olympia, WA. 57 pp.

This is the Washington Department of Ecology plan for implementing the marine sediment quality task for the Puget Sound Ambient Monitoring Program (PSAMP) dictated by the Puget Sound Water Quality Authority (PSWQA). It utilizes the triad approach which includes measures of chemical concentrations, benthic infaunal analyses and sediment bioassays. 119 Puget Sound stations were identified for annual sampling with most located along the 20-m depth contour. For sediment bioassays, 3 assay types are to be conducted:

Amphipod (*Rhepoxynius abronius*): 10-day exposures in 250 ml of sediment, 20 amphipods/chamber, 5 reps/sample, 15 ± 0.5 °C, salinity $28 \pm 1\%$, DO > 5 mg/liter, pH 8 ± 1 , interstitial salinity $> 25\%$. Two reference toxicants required for calculation of 96-hr LC50s (without sediments). Control survival of $\geq 90\%$ required with recordings of daily emergence. Sediment holding time = 14 days at 4 °C in the dark.

Bivalve larvae (*Crassostrea gigas* or *Mytilus edulis*): 48-hr exposures, 20 g sediment/chamber, 5 reps/sample, 20 ± 1 °C, salinity $28 \pm 1\%$, DO > 4 mg/liter, pH 8 ± 1 . LC50s for two reference toxicants required. Seawater and sediment control survival must be $\geq 70\%$ and $\geq 90\%$ normal development. Interstitial salinity $> 10\%$. Endpoints = 48-hr survival and abnormality measures

Microtox: Species possible use of organic and/or saline extracts for determining 15-min EC50s. For organic extracts, 500 g samples with 10 ± 0.5 g pre-extract volume. Sediment storage

<6 months at -20 °C. One reference toxicant required, 5 reps/sample with 2 dilution series. For saline extract, 200 g samples with 30 g pre-extract volume, 5 reps/sample with 2 dilution series. Sediment storage < 14 days at 4 °C and one reference toxicant required.

This plan also gives details of QA/QC requirements and reporting of data.

Swartz, R. C. 1984. Toxicological methods for determining the effects of contaminated sediments on marine organisms. Chapter 14, pp. 183-198 *In: Fate and Effects of Sediment-bound Chemicals in Aquatic Systems*, K. L. Dickson, A. W. Maki and W. A. Brungs, eds. SETAC Special Publ. Series, Pergamon Press, New York, NY.

This article reviews the state of sediment assays as of 1984. It gives a review table of sediment bioassays with numerous species and response criteria and an extensive set of references. Discusses factors related to test species selection, response criteria, experimental design, field validation and research priorities.

This review indicates that groups such as *Macoma*, *Nereis* and *Glycinde* are relatively insensitive to toxicants and should not be used in acute tests. Sensitive groups identified from field work in Southern California = Phoxocephalid and Ampeliscid amphipods, brittlestars and the polychaetes *Sthenelanella* and *Phoronis*. The authors suggest the use of a short life-cycle nematode bioassay.

Research needs:

- 1) Determine relationship between sediment toxicity and bioavailability
- 2) Importance of interactions between contaminants
- 3) Functional significance of sediment toxicity, particularly in relation to trophic dynamics and benthic-pelagic coupling.

Tetra Tech, Inc. and EVS Consultants, Inc. 1986. Recommended protocols for conducting laboratory bioassays on Puget Sound sediments. Final Rpt. TC-3991-04 for the U. S. Environmental Protection Agency, Region X, Puget Sound Estuary Program by Tetra Tech, Inc., Bellevue, WA. 55 pp.

This is the EPA Protocol Manual for conducting sediment bioassays in the Puget Sound region. It is the first such manual and will be periodically modified to reflect changes due to research findings or regulatory needs. The included bioassays were generally selected for their sensitivity and past usage in the Puget Sound region. Other promising methods are reviewed with some being identified for possible inclusion in the Manual. The Protocol Manual includes sections on sediment sampling, homogenization, storage and QA/QC guidelines.

The following tests are included in the Protocol Manual:

Amphipod (*Rhepoxynius abronius*): 10-day exposure test with survival and emergence as test endpoints. This test uses 175 g sediment in a total volume of 950 ml in 1-liter glass beakers at 15 ± 1 °C and 28 ± 1 % salinity, with aeration, sediments equilibrated overnight prior to addition of 20 amphipods, 5 replicates, constant light.

Bivalve larvae (*Crassostrea gigas* or *M. edulis*): 48-hr exposure test with mortality and abnormality endpoints. Temperature = 20 ± 1 °C, salinity 28 ± 1 ‰, container = 1 liter glass bottle, 20 g sediment in 1 liter total volume, shake 10 sec, add embryos & let settle, no aeration, 14:10 hr light:dark photoperiod. Use of 38-µm mesh Nytex screen for filtration at termination is OK (contrary to findings of Cardwell which modified the Woelke (1972) protocol to exclude this step) or decant beakers and subsample 10 ml directly. The protocol acknowledges the possible loss of embryos/larvae due to entrainment in the bottom sediments. Seed beakers with 20,000-40,000 embryos/beaker with "time zero" counts from the control seawater beakers for later mortality calculations.

Anaphase aberration: Specifies the use of rainbow trout gonad cell culture (RTG-2) using exposures to organic extracts of sediments but indicates that the test can be done with a variety of cell types. Sediments are frozen prior to extraction. Gives detailed extraction and testing procedures.

Microtox: Specifies procedures for both organic and saline extracts but emphasizes use of organic extracts until more data are available on saline extracts. Gives detailed procedures as per the standard Microtox methodology.

CHAPTER 2. AMPHIPOD BIOASSAYS

METHODOLOGY

Swartz, R. C., W. A. DeBen, J. K. P. Jones, J. O. Lamberson and F. A. Cole. 1984. Phoxocephalid amphipod bioassay for marine sediment toxicity. Pp. 284-307 In: Aquatic Toxicology and Hazard Assessment: Seventh Symposium, ASTM STP 854, R. D. Cardwell, R. Purdy and R. C. Bahner, eds. Am. Soc. Testing and Materials, Philadelphia, PA.

This is the presently accepted protocol for conducting amphipod, *Rhepoxynius abronius*, bioassays of marine sediments. This protocol is currently (1990) under review by ASTM (Sediment Toxicity Subcommittee) for acceptance as a standard ASTM protocol.

This article provides information on the relevance of an amphipod bioassay, required bioassay facilities, *R. abronius* life history and collection information, test response criteria, effects of sediment grain size, starvation, salinity, TVS and temperature. It also describes the results of previous field validation tests and reports that *R. abronius* survives TVS concentrations up to 18.2%, but that there was some mortality at 39.8%.

Protocol details:

Test species = *R. abronius*; 10-day exposures to 175 grams of sediment in 1-liter glass beakers to which 950 ml seawater is added. 20 amphipods/beaker, 5 replicates/sample preferred, with aeration at 15 ± 1 °C and salinity = 25 ± 1 ‰. The appendix gives "cook book" details on how to run this test.

Swartz, R. C., F. A. Cole, D. W. Schults and W. A. DeBen. 1986. Ecological changes in the Southern California Bight near a large sewage outfall: Benthic conditions in 1980 and 1983. *Marine Ecol. Progress Series* 31:1-13.

Sediment samples were collected in 1980 and 1983 (during a period of decreasing contaminant inputs) at 1-15 Km away from Los Angeles County sewer outfalls and at a reference station 47 Km distant, all at the 60-m depth contour. Samples were measured for chemicals, toxicity via amphipod (*Rhepoxynius abronius*) bioassays, and infaunal analyses. Amphipod tests = Swartz 10-day mortality endpoint.

Results:

- 1) Most parameters of chemical contamination decreased between 1980 and 1983, especially closest to the outfalls.
- 2) Stations closest to the outfalls were less dominated by *Capitella* and other "pollution resistant" species in 1983. Cluster groups of animals near the outfalls were distinctive for major degradation. Pollution-sensitive species of amphipods and echinoderms were absent. Other more distant stations showed various degrees of degradation or stimulation. The reference station was dominated by brittle stars.
- 3) Amphipod bioassays showed toxicity in sediments from 3 stations closest to the outfalls in 1980 but no toxicity at any station in 1983.

SEDIMENTS

Anderson, J. W. 1985. Biological and chemical analyses for the design and construction of the U. S. Navy Homeport Facility at East Waterway - Everett, Washington. Final Rpt. for Seattle District, U. S. Army Corps of Engineers by Battelle Pacific Northwest Marine Laboratory, Sequim, WA. 33 pp. + appendix. This report also appears in the FEIS, Carrier Battle Group, Puget Sound Region Homeporting Project (1985).

This study used the "Swartz Protocol" amphipod (*Rhepoxynius abronius*) bioassay to test sediments from the East Waterway, Everett. It used 10-day exposures to the solid phase, 20 animals/beaker, 4 reps/sample (not enough animals for 5 replicates - animals had to be ordered from Oregon due to sparse numbers at West Beach). No data given on test temperatures, salinities, pHs, DOs, amounts of sediments tested, sediment holding times, etc.

Results:

Used two test runs. Control (habitat) sediment mean survivals = 96.5 and 98.5%. Sequim Bay reference sediment survival = 82.5 and 63.0%. Everett test sediment survivals = 60.0 to 82.5%. End-of-test 1-hr reburial success data also recorded. "Bioassay results with the amphipod *Rhepoxynius abronius* did not seem to correlate with the bioaccumulation data or the sediment contaminant analyses."

DeWitt, T. and R. Swartz. 1987. An estuarine sediment toxicity test using infaunal amphipods. Paper presented at the Soc. for Environ. Toxicol. and Chem. (SETAC), 8th Annual Meeting, Pensacola, Fla., Nov., 1987.

The authors determined the sensitivity of *Eohastorius estuarius* to salinity, sediment particle size and a toxicant (fluoranthene) and compared it's sensitivity to these factors with that of *Rhepoxynius abronius* and the freshwater amphipod, *Hyalella azteca*.

Methods:

All experiments used the general protocol of Swartz et al. (1985): 10-day static exposure of 20 amphipods per 1 liter beaker with 2 cm sediment, constant aeration and light at 15 °C. Replication was 1 to 5 depending on the experiment. Salinity range tested = 2 to 28 %o, fluoranthene concentration = 1.1 to 40 mg/Kg, and a gradation of 42 intertidal and subtidal sediments were tested for grain size effects.

Results:

E. estuarius was insensitive to salinities as low as 2 %o and was slightly more sensitive to fluoranthene at 2 %o (LC50 = 13.8 mg/liter) than *H. azteca* at 20 %o (LC50 = 21.2 mg/liter) and slightly less sensitive than *R. abronius* at 28 %o (LC50 = 6.6 mg/liter). *E. estuarius* was insensitive to sediments of all grain sizes while *R. abronius* was slightly, but significantly, sensitive to fine-grained sediments.

Based on these results, *E. estuarius* is an excellent candidate for sediment bioassays because of its low salinity and grain size tolerances and its year-round availability from northern California to B. C., Canada.

DeWitt, T. H., G. R. Ditsworth and R. C. Swartz. 1988. Effects of natural sediment features on survival of the phoxocephalid amphipod, *Rhepoxynius abronius*. *Marine Environ. Res.* 25:99-124.

This study exposed amphipods to varying degrees of sediment grain sizes (without toxicant contamination) and water contents. The results of these tests were used to reinterpret the results of many previous test results of Puget Sound sediment/amphipod bioassays.

Methods:

Natural sediments were collected from Dabob Bay, Hood Canal, WA and sorted into several grain sizes by water elutriation. Amphipods, *Rhepoxynius abronius*, were exposed to these sediments of varying grain size and water content. Exposures were ala the "Swartz Method" (Swartz et al. 1985) with 10-day exposures to 2 cm of sediment in 1-liter glass beakers, 28‰ salinity, 15 °C, constant light with aeration. Control sediment = Yaquina Bay native sand.

For the reanalysis of Puget Sound field survey data, 127 reference and 170 urban bay sediment bioassay tests were utilized (the tests were conducted by 3 different labs).

Results:

Survival of amphipods in fine sediments was significantly less than for coarse and native sediments. Survival decreased slightly as the sediment water content increased from 57% to 72% and increased slightly from 72% to 80% water content. There were no differences in amphipod survivals in sediments stored at 4 °C for 7 days vs. 14 days.

Particle size alone cannot account for all toxicity seen in clean natural sediments. Handling of sediments also produces toxicity, possibly due to the release of "mineral particles." An equation is given for potential adjustment of future toxicity measures of fine-grained natural sediments.

Kemp, P. F. and R. C. Swartz. 1988. Acute toxicity of interstitial and particle-bound cadmium to a marine infaunal amphipod. *Marine Environ. Res.* 26:135-153.

The authors exposed amphipods, *Rhepoxynius abronius*, to cadmium in a flow-through exposure system to determine if toxicity was primarily a factor of interstitial cadmium concentrations or total cadmium bound to the sediments. The amount of total cadmium in the sediments was controlled by varying the amounts of fines (essentially organic) added to the control sediments. Interstitial water cadmium concentrations were controlled by the flow-through system which forced the water/cadmium flows through the sediments.

Methods:

Amphipods and sediments were collected from Yaquina Bay, Oregon. Bioassays generally followed the protocol of Swartz et al. (1985). The tests used 1-2 cm of sediment in 1 liter glass beakers modified for a flow-through system by cutting off the bottoms and replacing with screen. The beakers were set in a water-jacket flow-through system that forced the water/toxicant flows

through the sediments. Test temperature = 15 °C, salinity = 25 ‰, with aeration, 4-day exposure times, constant light, 20 amphipods/beaker with replication of 4-6 beakers/concentration. Test endpoints = survival and reburial success.

Results:

Survival and reburial success of amphipods in two experiments were proportional to the interstitial water cadmium concentrations and were not influenced by the total (bound + interstitial) cadmium concentrations (70-80% of the mortality of past tests could be predicted from the dissolved cadmium concentrations). Cadmium LC50s in these tests were in the range of 1.8 to 2.2 mg/liter dissolved cadmium. The reburial EC50s were ~1 mg/liter dissolved cadmium.

Mearns, A. J., R. C. Swartz, J. M. Cummins, P. A. Dinnel, P. Plesha and P. M. Chapman. 1985. Inter-laboratory comparison of a sediment toxicity test using the marine amphipod, *Rhepoxynius abronius*. *Marine Environ. Res.* 19:13-37.

Five bioassay laboratories participated in an interlaboratory assay of contaminated and cadmium-amended sediments using the amphipod *R. abronius* and the protocol of Swartz et al. (1985). Four *a priori* and one *a posteriori* hypotheses were tested concerning the success of the inter-lab comparison.

Methods:

Five labs conducted tests in parallel and cooperated in planning and pre-test logistics and collection of animals and sediments. Test protocol = 10-day static test in 1 liter glass beakers with 175 grams (2 cm) of sediment, constant aeration and light, 15 ± 1 °C and ≥ 25 ‰ salinity. Each beaker was seeded with twenty 3-5 mm amphipods with 5 replicate beakers/sediment sample. Cadmium chloride at 3 concentrations was used as a toxic control. The control sediment was from West Beach. Test endpoints = survival, emergence during the test and reburial success at the end of the test. Test sediments were stored at 4 °C for <14 days.

Results:

Temperature, salinity, pH and DO were satisfactory in all test beakers in all labs. Control survivals were all >90%. Cadmium LC50s ranged from 9.44 to 11.45 mg/Kg for the 5 labs with a grand mean of 9.81 mg/Kg. Three of four *a priori* hypotheses were satisfactorily met including : 1) acceptable control responses, 2) toxicity ranking of sediments, and 3) inter-lab agreement on mean responses. The one *a priori* hypothesis not met was classification of samples as toxic or non-toxic as compared to the controls. The labs did agree on clearly toxic and non-toxic samples, but did not agree when toxicity was marginal (i.e., 76-87% survival).

Plesha, P. D., J. E. Stein, M. H. Schiewe, B. B. McCain and U. Varanasi. 1987. Toxicity of marine sediments supplemented with mixtures of selected chlorinated and aromatic hydrocarbons to the infaunal amphipod *Rhepoxynius abronius*. Manuscript accepted for publication in *Marine Environ. Res.* (probably in 1988).

This study exposed amphipods to natural marine sediments supplemented with mixtures of 7 aromatic hydrocarbons (AHM) or 4 chlorinated hydrocarbons (CHM) at concentrations

simulating Puget Sound contaminated sediments (= 1X) or 5 times higher than Puget Sound concentrations (= 5X). These mixtures were also radio-labeled to track retention in the test sediments and uptake by the amphipods.

Methods:

Natural sediment was collected by van Veen grab from the Dosewallips River, Hood Canal area and stored for 48 hours at 4 °C. Hydrocarbons were added to this sediment by mixing 500 g of sediment with 200 ml seawater (5 μm -filtered) for 48 hours, followed by washing and filtering twice with filtered seawater. The hydrocarbons were added in small amounts of acetone.

Amphipods were collected from West Beach, Whidbey Island. The protocol of Swartz et al. (1985) was used for the bioassays except that the amount of sediment in each beaker was 50 ml. Exposures were for 10 days at 28 ‰ salinity, 15 °C, constant light and aeration, 20 amphipods/beaker and 5 replicates (+ 2 replicates for chemical analyses). Test endpoints = survival and reburial success.

Results:

Generally, sediment concentrations of hydrocarbons remained fairly stable during the 10 day tests. Significant reductions in survival of *R. abronius* occurred in the 1X and 5X CHM sediments and in the 5X AHM sediments. Amphipods also showed an impaired ability to rebury following exposures to CHM sediments. There was also a dose-dependent uptake of radio-labeled compounds by amphipods in all exposures, which also corresponded to the toxicity results.

Robinson, A. M., J. O. Lamberson, F. A. Cole and R. C. Swartz. 1988. Effects of culture conditions on the sensitivity of a phoxocephalid amphipod, *Rhepoxynius abronius*, to cadmium in sediment. Environ. Toxicol. Chem. 7:953-959.

This work investigated the feasibility of culturing *R. abronius* in the laboratory and using these animals for routine bioassay tests for toxicity. The various experiments evaluated the subsequent sensitivity of cultured amphipods to cadmium relative to handling stresses, juveniles vs. adults and cultured vs. wild animals.

Methods:

The culture system used medium-sized culture trays held in a continuous-flow seawater system (1.2-1.5 liters/min) with salinities = 27-31 ‰, temperature = 8-13 °C and 2 cm depth of native sand from Yaquina Bay, Oregon. Bioassays were conducted using the protocol of Swartz et al. (1985) with 2 cm sediment in 1 liter glass beakers, with constant aeration and light, 25 ‰ salinity, 15 °C, 10-day exposures, no feeding and 20 amphipods/beaker with 5 replicates/concentration.

Results:

Amphipod survival in the long-term culture system was greater for 2 mm vs. 1 mm amphipods, with 48-56% survival at 180 days for the 1 mm animals and 71-83% for 2 mm animals. Sieving of the amphipods had no effect on their sensitivity to cadmium, but the cultured animals (LC50 = 4.4 mg/liter) were more sensitive than wild animals (LC50 = 8.7 mg/liter) to

cadmium. The juveniles were also more sensitive (LC50 = 8.2 mg/liter) than adult amphipods (LC50 = 11.5 mg/liter).

The authors recommend that wild adult amphipods (size = 3-5 mm and used within 14 days of collection) to minimize variables associated with inherent natural biological variability.

SCCWRP. 1989. Influence of sediment type on phenanthrene toxicity. Pp. 62-65 *In: South. Calif. Coast. Water Res. Proj. Annual Report 1988-1989*, Long Beach, CA.

This study investigated the toxicity of clean sediments spiked with phenanthrene, a common PAH contaminant of sediments in Southern California, to the amphipod *Grandidierella japonica*.

Methods:

Two clean natural sediments of varying grain size and TOC (Newport Bay = 97% sand/0.1% TOC and San Mateo Point = 95% silt/clay and 1.0% TOC) were spiked with phenanthrene concentrations of 0, 10, 30 and 90 mg/kg (nominal).

Amphipods were exposed to a 2-cm layer of sediments for 14 days in a flow-through system at 20° C. Some phenanthrene was labeled with ¹⁴C to follow its fate. Phenanthrene concentrations were measured with GC/MS and LSC.

Results:

Roughly half of the added phenanthrene in sediments was degraded to other chemical forms, possibly by microorganisms. 14-day amphipod survivals in all concentrations were not significantly different from the controls, but animals in the 90 mg/kg concentration showed only about 50% survival. Interstitial water phenanthrene concentrations were always higher in the Newport Bay (low TOC) sediment, leading to higher amphipod body burdens in this sediment. Thus, toxicity and uptake was primarily via the interstitial water concentrations. The 14-day LC50 for *G. japonica* was >30 mg/kg (adjusted for availability) vs. *Rhepoxynius abronius* with a 10-day LC50 of 3.7 mg/kg (Swartz et al. 1989, *Environ. Toxicol. Chem.* 8:215-222).

Swartz, R. C., W. A. DeBen and F. A. Cole. 1979. A bioassay for the toxicity of sediment to marine macrobenthos. *J. Water Poll. Cont. Fed.* 51(5):944-950.

This is a report on one of the earliest sediment bioassays conducted by the EPA/Newport Research Laboratory. Five benthic invertebrate species were tested in flow-through solid-phase assays that tested sediment depth, grain size effects and a range of contaminated sediments from various U. S. marine locations.

Bioassay species tested were:

Littleneck clam, *Protothaca staminea*

Clam, *Macoma inquinata*

Polychaete, *Glycinde picta*

Amphipod, *Paraphoxus epistomus* (= *Rhepoxynius abronius*)

Cumacean (used four species)

Basic test design:

Flow-through tests (0.5 liters/min) in 25 liter polyethylene boxes at ambient temperature (9.5-16.8 °C) and salinity (23.5-32.7 ‰), 20 individuals/box, 28 mm of control sediment topped by 15 mm of test sediment following a 48-hour acclimation period for animals added to the control layer. All test exposures were 10 days with survival as the endpoint.

Results:

Burial tests: Burial layers 5 to 50 mm thick were tested (on top of 28 mm of control sediment). No significant difference in survivals at any depth. 15 mm sediment depth was selected for routine testing.

Grain size tests: Silts to very coarse sands were tested for effects. The only obvious reduction in survival was for the cumaceans in the coarse to very coarse sands.

Sediment toxicity tests: Sediments from the Skipamon River and Coos Bay, Oregon were non-toxic in all tests. Some sediments from the Raritan River, NJ, Bailey Creek, VA, Houston Ship Canal, Duwamish River and Elliott Bay, Puget Sound were all toxic in various degrees. The most toxic sediment was from the Houston Ship Canal.

Relative organism sensitivity (from least to most sensitive): *Protothaca* < *Macoma* < *Glycinde* < *Paraphoxus* < *Cumacea*.

Swartz, R. C., D. W. Schults, G. R. Ditsworth and W. A. DeBen. 1984. Toxicity of sewage sludge to *Rhepoxynius abronius*, a marine benthic amphipod. *Arch. Environ. Contam. Toxicol.* 13:207-216.

This study involved the addition of sewage sludges to clean marine sands to determine the relationship between amphipod mortality and total volatile solids (TVS) and/or toxic contaminants. Sludges were collected from two Oregon community treatment plants, two of the major Los Angeles treatment plants and from one plant each in New York City and New Jersey.

Methods:

Sludges were mixed with Yaquina Bay, Oregon sands to produce TVS concentrations of 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0% above background. Bioassays were 10-day exposures to 175 grams (2 cm) sediment in 1 liter glass beakers, temperature = 15 °C, salinity = 25 ‰, DO ranged from 7.0 to 9.7 mg/liter and pH = 7.8-9.0. All beakers were aerated.

Results:

Sludge Source	LC50 (as % TVS)
Waldport, Oregon	2.83
Newport, Oregon	0.28
Los Angeles City	0.08
Los Angeles County	0.07
Staten Island, New York	0.44
Middlesex, New Jersey	0.42

The authors concluded that "the great differences between sludge sources in *Rhepoxynius abronius* mortality at comparable levels of TVS addition clearly indicates that toxicity is related to chemical contamination rather than organic enrichment." Twenty-two of 90 rank correlations for toxicity vs. chemical contamination were statistically significant. Highest correlations for amphipod mortalities ($\geq 50\%$) were with: Hydrocarbon oil and grease, total oil and grease, zinc, cadmium, Eh, nickel and ammonia.

Swartz, R. C., D. W. Schults, G. R. Ditsworth, W. A. DeBen and F. A. Cole.
 1985a. Sediment toxicity, contamination and macrobenthic communities near a large sewage outfall. Pp. 152-175 In: Validation and Predictability of Laboratory Methods for Assessing the Fate and Effects of Contaminants in Aquatic Ecosystems, T. P. Boyle, ed. ASTM STP 865, Am. Soc. for Testing and Materials, Philadelphia, PA.

This investigation tested the relationship between sediment chemistry, benthic infaunal indices and sediment bioassays with amphipods for sediments collected along a 7-station gradient of sewage contamination off Los Angeles, CA.

Methods:

Physical/chemical analyses: Analyses included total solids, TVS, TOC, 5-day BOD, total oil and grease, hydrocarbon oil and grease, sulfide, metals, grain size, and priority organic pollutants.

Benthic infaunal analyses: Five replicate van Veen grab samples were collected from each station and sieved through 1.0-mm screens and processed for all species.

Amphipod bioassay: 10-day *Rhepoxynius abronius* assays *ala* Swartz et al. (1985) method. 2 cm sediments in 1 liter glass beakers, 25‰ salinity, 15 °C, with constant aeration and photoperiod, and 5 reps/station

Results:

Macrobenthos numbers, biomasses and diversities were depressed at the 3 stations closest to the outfall and stimulated at intermediate stations. Chemical indices indicative of pollution were most elevated at the 3 stations closest to the outfall. Significant amphipod mortalities were observed only in sediments collected from the stations closest to the outfall. "Statistically significant rank correlations occurred between sediment toxicity and 18 biological and geochemical parameters including phoxocephalid density, total amphipod density, and various measures of organic enrichment and chemical contamination."

Swartz, R. C., G. R. Ditsworth, D. W. Schults and J. O. Lamberson. 1985b.
 Sediment toxicity to a marine infaunal amphipod: Cadmium and its interaction with sewage sludge. *Marine Environ. Res.* 18:133-153.

This work exposed amphipods, *Rhepoxynius abronius*, to cadmium in seawater, cadmium in sediments and to cadmium in sediments with natural organics and sewage sludge added to test sediments.

Methods:

All bioassays generally followed the protocol of Swartz et al. (1985). Sediments and amphipods were collected from Yaquina Bay, Oregon and sewage collected from a municipal plant in Waldport, OR. Twenty adult (4 mm) amphipods/1 liter glass beaker with 2 cm sediment, constant aeration and light, 25 ‰ salinity, 15 °C, and DO = 9.3 to 9.9 mg/liter. Exposure times were 10 days for sediments and 96 hrs for water-borne tests. Toxicant = cadmium chloride dissolved in 25 ‰ seawater. Cadmium and organics were added to sediments in rolling jars. Sediments were stored <14 days at 4 °C. Five replicates/test concentration. Test endpoints = survival, emergence during the test and post-test reburial success.

Results:

The LC50s and EC50s in mg/liter as cadmium ion were (NT = not tested):

Test Type	Exposure Time (days)	LC50	Reburial EC50
Cd in sediment	10	6.9	6.5
Cd in sediment	4	25.9	20.8
Cd in seawater	4	1.61	0.55
Cd in interstitial water			
Day 0 concentration	4	3.64	NT
Day 4 concentration	4	1.42	NT

Interstitial water contained only 1.7 to 4.4% of the total cadmium added to the sediments. Enrichment of the sediments with sewage sludge or Yaquina Bay "fines" substantially decreased the toxicity of cadmium in sediments, probably due to the physical binding of the Cd by particulates, thereby reducing bioavailability. These tests indicated that essentially all of the toxicity in the Cd/sediment exposures was probably due to the interstitial water Cd concentrations and not due to Cd bound to the sediment particles.

Swartz, R. C., D. W. Schults, T. H. DeWitt, G. R. Ditsworth and J. O. Lamberson. 1987. Toxicity of fluoranthene in sediment to marine amphipods: A test of the equilibrium partitioning approach to sediment quality criteria. Paper presented at the Soc. of Environ. Toxicol. and Chem. (SETAC), 8th Annual Meeting, Pensacola, Fla., Nov. 1987. Manuscript also submitted to Environ. Toxicol. and Chem. for publication.

The authors exposed amphipods, *Rhepoxynius abronius*, to fluoranthene at three concentrations of total organic carbon (TOC) to explore resulting toxicity patterns with an equilibrium partitioning model.

Methods:

R. abronius sediment assays were conducted following the basic protocol of Swartz et al. (1985): 10 day exposures in a static system with 2 cm sediments in 1 liter glass jars with constant aeration and light, 20 amphipods/jar and 2 replicates/sediment. Fluoranthene concentrations = 0.2, 0.3, and 0.5 g/dry KG/10 (?).

Results:

LC50s as bulk fluoranthene concentrations = 3.3, 6.2 and 10.5 mg/liter for TOC concentrations of 0.2, 0.3 and 0.5 g/dry KG/10, respectively. LC50s as interstitial water concentrations = 22.0 to 31.1 mg/liter with no trend observed relative to TOC content.

The authors concluded that acute toxicity was primarily associated with fluoranthene dissolved in the interstitial water and that interstitial water concentrations were similar to those predicted by an equilibrium partitioning model.

Swartz, R. C., P. F. Kemp, D. W. Schults and J. O. Lamberson. 1988. Effects of mixtures of sediment contaminants on the marine infaunal amphipod, *Rhepoxynius abronius*. Environ. Toxicol. Chem. 7:1013-1020.

This work exposed amphipods to lab-spiked sediments of 4 single toxicants and those toxicants in combinations of 2, 3 or 4 in order to determine if toxicity of toxicant combinations were additive, antagonistic or synergistic.

Methods:

Amphipods were exposed to single toxicant or toxicant combinations in 2-cm deep layers of sediments in 1-liter beakers for 10 days at 15 °C, 25 % salinity, with constant light and aeration and no food. Toxicant/sediment mixtures were prepared in rolling mill jars. Toxicants were zinc, mercury, fluoranthene and PCB (Aroclor 1254). The first experiments determined single toxicant LC50s. The combination experiments used concentrations of 1/2 the LC50 for each chemical. Tests were conducted at two TVS concentrations: 1.72 and 1.30%.

Results:

Amphipod 10-day LC50s (μg/g, dry wt) were:

Zinc	=	276
Mercury	=	13.1
PCB	=	10.8
Fluoranthene	=	4.2

For combination experiments, there was a direct relationship between mortality and number of chemicals present (each at 1/2 of their LC50 concentrations). Mean mortalities for the combinations were:

1 chemical	=	11%
2 chemicals	=	35.5%
3 chemicals	=	60%
4 chemicals	=	87%

Mortalities were significantly greater at 1.30% TVS vs. 1.72% TVS. The authors concluded that, for these chemicals, joint action was additive or less than additive and suggest that sediment quality criteria based on individual "safe" concentrations are not adequate when multiple contaminants are present.

Swartz, R. C., P. F. Kemp, D. W. Schults, G. R. Ditsworth and R. J. Ozretich.
1989. Acute toxicity of sediment from Eagle Harbor, Washington to the infaunal amphipod *Rhepoxynius abronius*. *Environ. Toxicol. Chem.* 8:215-222.

Amphipod bioassays were conducted on 3 sediment samples collected in June 1985 from Eagle Harbor, Washington (off Wycoff Plant). Sediment samples were collected by 3-4 van Veen grabs (10-17 cm deep) at each station and the sediments composited. Sediments were stored at 4 °C for 2-30 days.

Methods:

Four-day amphipod exposures to the solid phase with 2-cm depth in 1-liter beakers, 775 ml seawater at 15 °C, 28 ‰ salinity, 20 amphipods/beaker, 3 reps/sample. Also tested interstitial water dilutions with amphipods. Sediments centrifuged at 4,000 RPM at 5 °C for 10 min and filtered through 1-µm glass-fiber filters. Test solutions = 0.25 to 4% interstitial water in 28 ‰ Yaquina Bay seawater. Also, a dilution series of the Eagle Harbor solid phase was tested with sediment concentrations of 269 to 1,600 mg/Kg in Yaquina Bay sand.

Results:

One Eagle Harbor sediment sample was very toxic (total PAH concentration = 6,416 mg/Kg) with total mortality of amphipods in sediment concentrations >1,120 mg/Kg and in 4% interstitial water. Solid-phase LC50 = 666 mg/liter (~2.59 mg/Kg PAHs) and the LC50 for interstitial water = 0.89%. Mean 4-day survivals in the solid phase from the remaining two stations (each within 150 m of the toxic station) = 87 and 98%. All control survivals were >90%.

The toxic Eagle Harbor sediment was by far the most acutely toxic sample tested to date in the U. S. The total concentration of PAHs (6,461 mg/Kg) was way less than in other Eagle Harbor samples (up to 29,000 mg/Kg). This paper discussed the possibility that most of the toxicity of the solid phase was due to the interstitial water PAH concentrations.

MISCELLANEOUS

Kemp, P. F., F. A. Cole and R. C. Swartz. 1985. Life history and productivity of the phoxocephalid amphipod *Rhepoxynius abronius* (Bernard). *J. Crustacean Biol.* 5(3):449-464.

The life history of *R. abronius* was studied over a period of slightly more than 1 year (1980-1981) in Yaquina Bay, Oregon. The collection site was a sandy channel at ~ 5 meters depth. Natural temperature and salinity ranges were 8-13 °C and 22-33 ‰, respectively.

Information is provided on size-frequency, size at maturity, reproduction, growth and maturation, biomass, recruitment, seasonality, longevity, productivity and mortality. A summary chart of the major life-history events is also given.

CHAPTER 3. EMBRYO/LARVAL BIOASSAYS

METHODOLOGY

APHA (American Public Health Association). 1980. Bioassay procedures for mollusks (tentative). pp. 715-722 *In: Standard Methods for the Examination of Water and Wastewater*, 15th edition. APHA, Washington, D. C. 1134 pp.

This is a rather general guide for conducting a variety of tests (fertilization, embryo/larval development, growth, byssal thread secretion, etc.) with about 10 species of oysters, mussels, clams and scallops. It briefly covers water supply and lab requirements, test animal collection, conditioning, feeding and spawning for each group and the conduct of each type of bioassay.

ASTM (American Society for Testing and Materials). 1980. Standard practice for conducting static acute toxicity tests with larvae of four species of bivalve molluscs. Designation E 724-80, ASTM Annual Book of ASTM Standards, Philadelphia, PA. 17 pp.

This article provides a Standard Practice guide for conducting embryo/larval assays with four species:

Eastern oyster, *Crassostrea virginica*
 Pacific oyster, *Crassostrea gigas*
 Bay mussel, *Mytilus edulis*
 Quahog clam, *Mercenaria mercenaria*

The testing protocol follows that of Woelke (1972) very closely. This protocol covers the following topics: Test significance, terminology, test materials, dilution water, toxicant preparation, test species collection, conditioning, spawning, test design, data analyses and reports. Stipulates that control mortality must be <30% and that control abnormality must be <10%.

Cardwell, R. D., C. E. Woelke, M. I. Carr and E. Sanborn. 1978. Variation in toxicity tests of bivalve mollusc larvae as a function of termination technique. *Bull. Environ. Contam. Toxicol.* 20:128-134.

The authors looked at the effect of filtration of oyster (*Crassostrea gigas*) and horse clam (*Tresus capax*) embryos through 37 μm mesh (diagonal distance = 62 μm) Nytex screen at the end of the bioassays. Also tested toxicity of cadmium sulfate, dodecyl sodium sulfate and methoxychlor on both species as well as natural marine waters from Puget Sound. Used 48-hr test exposures, dissolved oxygen = 6.8 mg/liter, pH = 7.8, salinity = 29 ‰ for the lab dilution/control water.

Results:

Quality of the oyster gametes was better than for the horse clams (probably more experienced with oyster conditioning). Filtration through Nytex apparently allowed loss of smaller, probably abnormal, larvae. This tends to cause an increase in the apparent mortality and a decrease

in the apparent abnormality. Thus, the filtration step in the Woelke (1972) protocol should be eliminated in favor of direct sampling from the beakers following mixing.

The authors suggested the consideration of combining mortality and abnormality into one measure of "ecological mortality" (see Legore 1974). Paper also gives LC50s and EC50s for the above named toxicants and natural seawater samples from Puget Sound.

Karnofsky, D. A. and E. B. Simmel. 1963. Effects of growth-inhibiting chemicals on the sand-dollar embryo, *Echinorachnius parma*. *Prog. Exp. Tumor Res.* 3:254-295.

This paper primarily provides an overview of the possible use of sea urchin gametes and embryos for research on drug effects, especially as related to cancer research. It covers in detail the biology of sand dollars, their gametes, embryonic development, morphology and biochemistry. The authors then cover the basic methodology for using gametes and embryos in testing programs, but at a rather general level. The authors suggest the use of plastic ice cube trays as exposure chambers with 10 ml of seawater. They then give some results (again, very general) of tests with various drug preparations (e.g., dinitrophenol, actidione, colchicine, BrUDR, etc.).

Included in the article are several pages of good photographs and graphs of development.

Chapman, P. M. and J. D. Morgan. 1983. Sediment bioassays with oyster larvae. *Bull. Environ. Contam. Toxicol.* 31:438-444.

The authors developed a modification of the oyster larval bioassay which incorporates sediments in the test containers. Sediments from 22 Puget Sound stations were tested with 48-hr Pacific oyster embryo/larval development. Temperature = 20 ± 1 °C, Salinity = 25 ‰, DO = 8 mg/liter (initial), pH adjusted to 8.0. Sediments were tested by adding 15 g to 750 ml seawater, rotating at 10 RPM for 3 hr, followed by embryo inoculation at 35 embryos/ml. No mixing during exposure. At 48 hr, samples processed by decanting bottles through 0.042 mm Nytex screen (this procedure leaves an unknown number of embryos behind that may be trapped in the sediments).

Results:

No absolute measure of seawater control mortality was made; rather, 48-hr seawater control survival set to 100% relative to the test sediments. Sediment controls had greater number of survivors relative to the seawater controls (134%). There was no discussion of this point. Survival in the test sediments generally agreed with data on abnormalities, although there was some variability. 13 of the 22 Puget Sound sediments were highly toxic, 5 samples moderately toxic and 4 non-toxic.

Dinnel, P. A. and Q. J. Stober. 1985. Methodology and analysis of sea urchin embryo bioassays. Circular No. 85-3, Fish. Res. Institute, University of Washington, Seattle, WA. 19 pp.

This article provides a step-by-step methodology for conducting sea urchin and sand dollar embryo/larval bioassays with 3 species of Northwest echinoids.

Recommended Northwest test species are:

1. Purple sea urchin, *Strongylocentrotus purpuratus*
2. Green sea urchin, *S. droebachiensis*
3. Sand dollar, *Dendraster excentricus*

Methodology covers:

1. Life cycles
2. Collection, handling and feeding
3. Spawning, fertilization and gamete quality
4. Bioassay procedure
5. Exposure conditions:
 - a. ~25 embryos/ ml seeding density
 - b. Replication ≥ 3
 - c. Salinity = $30 \pm 3 \%$
 - d. Temperature = $8-10^{\circ}\text{C}$ for urchins and $12-16^{\circ}\text{C}$ for sand dollars
 - e. Exposure time 48-96 hr depending on species and temperature
6. Sample analyses
7. Provides a good bibliography on various sea urchin tests.

Dimick, R. E. and W. P. Breese. 1961 (?). Bay mussel embryo bioassay. Pp. 165-175
In: I'm not sure but I think it's: Northwest Symposium on Water Pollution and Toxicity,
 U. S. Health Ed. and W.P.H.S., Reg. IX, 10:165-175.

This paper describes a suggested water quality assay using *Mytilus edulis* embryo/larval development in a 48-hr test. Very similar to an oyster embryo assay. Species spawning by addition of KCl to seawater and test salinities $\geq 25 \%$. Also, suggests the use of a stain to help detect normal vs. abnormal larvae. This paper also reports test results with Kraft Mill effluents, various chemical components of KME, Sevin, and NaPCP.

Esposito, A., M. Cipollaro, G. Corsale, E. Ragucci, G. G. Giordano and G. Pagano. 1984. The sea urchin bioassay in testing pollutants. *In: Strategies and Advanced Techniques for Marine Pollution Studies*, H. J. M. Dov and C. S. Giam, editors. NATO, Brussels, Belgium.

This is a general review paper that discusses the various types of tests possible as part of a "sea urchin test system" for marine pollution monitoring and other basic toxicological studies. This paper suggests a general protocol for conducting tests (no specific cook-book directions) for assessing fertilizing capacity of sperm, larval malformations and mitotic abnormalities. Also, it discusses advantages and disadvantages of the test system.

Hose, J. E. 1985. Potential uses of sea urchin embryos for identifying toxic chemicals: Description of a bioassay incorporating cytologic, cytogenetic and embryologic endpoints. *J. Applied Toxicology* 5 (4):245-254.

This report describes the use of a sea urchin embryo test (SET) which is based on a 48-hour development assay. Methods are identified for evaluation of embryotoxicity, teratogenicity and genotoxicity.

Developmental effects include: Fertilization success, abnormal development, retardation of development, cytolysis and mortality.

Cytologic effects include: Cytologic irregularities (pycnosis, karyolysis, karyorrhexis, abortive mitoses, pleomorphism, sticky chromosome bridges, dedifferentiation, premature differentiation and giant cell formation), anaphase aberrations (various measures) and micronucleus formation. This article includes many good illustrations of these abnormalities.

Test exposures to benzo(a)pyrene (BP):

Used 48-hour exposures of purple sea urchin (*Strongylocentrotus purpuratus*) gametes and embryos to 0.5 to 50 $\mu\text{g}/\text{liter}$ BP during fertilization and development to gastrula, at 15 $^{\circ}\text{C}$ with continuous stirring and aeration.

Results of BP tests:

No effects on fertilization success. Fewer embryos completed gastrulation at $\geq 1 \mu\text{g}/\text{liter}$ BP and they exhibited developmental abnormalities. Various genotoxic effects were noted at the lowest dose of 0.5 $\mu\text{g}/\text{liter}$ BP. Micronucleus induction and cytologic abnormalities were present only at BP concentrations $\geq 1.0 \mu\text{g}/\text{liter}$.

Loosanoff, V. L. and H. C. Davis. 1951. Delaying spawning of lamellibranchs by low temperature. *J. Marine Res.* 10(2):197-202.

The authors transferred oysters and clams from Long Island, NY to Maine waters (5-8 $^{\circ}\text{C}$ cooler) to retard gametogenesis during the summer months and thereby yield ripe animals in Sept and Oct after the normal spawn-out time in August.

After Oct/Nov, oysters (*Crassostrea virginica*) can be thermally conditioned to spawn during winter, but must wait until about Nov to condition because oysters must rebuild glycogen reserves following summer spawn-out. Oysters remain in fall spawning condition a shorter time than clams (*Venus mercenaria*).

Palermo, M. R. and E. L. Thackston. 1988. Refinement of column settling test procedures for estimating the quality of effluent from confined dredged material disposal areas. *Tech. Rpt. D-88-9, Environ. La., Waterways Experiment Station, U. S. Army Corps of Engineers, Vicksburg, MS.* 30 pp.

This paper describes a laboratory column settling test for sediments. It describes four types of settling dynamics and gives typical suspended particulate concentration profile diagrams through time. Typical fine sediments can take 24 hr or more for 90% particulate settlement, but rates depend on several variables.

This paper has data pertinent to factors involved with preparing elutriates for bioassay testing, particularly for larval assays of the suspended particulate phase.

Seno, H., J. Hori and D. Kusakabe. 1926. Effects of temperature and salinity on the development of the eggs of the common Japanese oyster, *Ostrea gigas*, Thunberg. J. Imp. Fish. Institute 22(3):41-47.

The authors conducted larval development tests with *Ostrea* (= *Crassostrea*) *gigas* at varying temperatures and salinities. Temperatures tested = 11.3-31.9 °C. Salinities tested = 1.0099-1.0274 (as specific gravities).

Results:

For temperature, high and low limits for development = 15 & 30 °C with an optimum range of 23-26 °C. Temperatures below about 23 °C greatly affected development times (to shell formation). For instance, 25.6 °C = 23 hr; 20.8° = 34 hr; and 16.3° = 83 hr.

For salinity, high and low limits for normal development = 1.0139-1.0246 as specific gravity. Optimum development (at 15 °C) = 1.017-1.021. Salinity had less effect on development time than temperature.

Woelke, C. E. 1972. Development of a receiving water quality bioassay criterion based on the 48-hour Pacific oyster (*Crassostrea gigas*) embryo. Tech. Rpt. No. 9, Washington Department of Fisheries, Olympia, WA. 93 pp.

Woelke developed and validated an oyster embryo assay for the State of Washington and proposed the following criterion for marine water quality:

"Where marine water uses include fish and/or shellfish reproduction, rearing, and/or harvesting, the per cent abnormal 48-hour Pacific oyster embryos shall not exceed 5% in 95% of the samples and under no circumstances exceed 20% in a single sample. The criterion shall not apply if the salinity is less than 20‰. If the bioassay control per cent abnormals exceed 3%, the per cent net risk statistic rather than the per cent abnormal may be applied."

Justification for this proposed criterion is based on 10 years of bioassay data and many studies which showed that if oyster embryo development in a 48-hr test is protected, then other life stages of oysters and clams as well as other marine animals will, in most cases, also be protected. Comparative studies include data on sulfite waste liquors (from paper mills), Sevin, NTA, DDT, sodium PCP, metals, NaSO₃, picric acid, tannic acid, phenol, several other pesticides, alcohol, acetone, chloroform, crude oil WSF and dispersants.

This report gives a detailed methodology of the oyster embryo bioassay with detailed discussion of many factors which may affect the conduct and results of the test. Variables considered include: adult oyster quality and conditioning, male/female gamete interactions, precision of culture sampling and reading, culture vessel size, embryo densities, age of water, age of larvae at termination, temperature, salinity, and field vs. lab tests. The major affective variables were: condition of the spawning adults, salinity, age of the water and temperature.

Many tables of data are included in the report to substantiate the various experiments and conclusions.

SEDIMENTS

Cardwell, R. D., C. E. Woelke, M. I. Carr and E. W. Sanborn. 1976. Sediment and elutriate toxicity to oyster larvae. Pp. 684-718 *In: Proceedings of the Speciality Conference on Dredging and its Environmental Effects*, P. A. Krenkel, J. Harrison and J. C. Burdick, III, eds. Am. Soc. Civil Engineers, New York, NY.

Pacific oyster, *Crassostrea gigas*, embryos were exposed for 48 hr at 20 °C to Grays Harbor sediments in a rotating jar system and to saline elutriates of those sediments. Also tested were artificial and "cleaned" natural sediments (homologues).

Methods:

Solid-phase tests: About 0.1 to 20 g sediment were added to 900 ml seawater in sealed polyethylene bottles at 20 °C, 29-30‰ salinity, pH = 7.4-7.8, DO >5 mg/liter and bottles rotated at 4 RPM for 48 hr. At end of test, water and sediment were mixed, sediments allowed to settle briefly and subsample aliquotes of embryos taken.

Elutriate Test: Various sediment amounts up to 1:4 sediment :seawater were prepared via "standard elutriate test" procedures as specified in EPA/COE (1977).

Results:

Seawater control mortality in two tests = 6.0 and 39.8%. Control abnormality = 0.3 and 1.0%.

For the solid-phase tests, mortality LC50s ranged from 0.4 to 5.8 g/liter and abnormality EC50s ranged from 0.7 to 16.8 g/liter. Most effects in the solid-phase tests were correlated with sulfides (~81%), oil & grease and volatile solids. Mechanical abrasion was also considered a major factor in the mortality of the embryos. Artificial sediment homologues also showed "toxicity" similar to the natural test sediments.

For the elutriate tests, there was low toxicity with most "no effect" concentrations >50% dilution of 1:4 elutriates. Low levels of copper, lead, nickel, and pesticides were detected in the elutriates but most of the toxicants appeared to be tightly bound to the sediments. The authors proposed that a better elutriate preparation procedure might eliminate the filtration step and use a high-speed continuous-flow centrifuge instead.

Carr, M. I. 1975. Bivalve embryo water quality monitoring bioassays of Olympia Harbor dredge spoil disposal. Pp. 27-44 *In: Evaluation of Effects of Dredging and Disposal on the Marine Environment in Southern Puget Sound, Washington*. Final Rpt. to Seattle District Corps of Engineers by Washington Department of Fisheries, Olympia, WA. 140 pp.

The author conducted 48-hr and 6-hr oyster (*Crassostrea gigas*) embryo bioassays of field-collected marine waters in the area of dredging in South Puget Sound (before, during and after dredging). Also used 48-hr Japanese littleneck clam (*Tapes semidecussata*) embryo assays when oysters continually failed the mortality criteria. There was a major problem with oyster embryo survival in a large number of samples during all bioassay runs. This problem precluded any possible attempts to define impacts due to the dredging activities. High mortalities were possibly

due to phytoplankton metabolites; however, embryo survival was not significantly correlated with chlorophyll A values (nor any of the other 13 measured parameters).

Note: Seawater controls were always set to 100% survival automatically, so that absolute mortalities in the controls were not reported.

Chapman, P. M. and J. D. Morgan. 1983. Sediment bioassays with oyster larvae. *Bull. Environ. Contam. Toxicol.* 31:438-444.

See this entry under **Methodology** for results of sediment tests with 22 Puget Sound sediments.

Davis, H. C. 1960. Effects of turbidity-producing materials in sediment on eggs and larvae of the clam (*Venus (Mercenaria) mercenaria*). *Biol. Bull.* 118:33-34.

This work tested effects of sediments on development of clam larvae using a rotating wheel to keep sediments suspended. The four sediment types tested were: Clay, Fullers earth, chalk and natural aquatic silt. Embryos were exposed for 48 hr and 12 days in 32-oz polyethylene bottles rotated at 8 RPM at 24 °C in 800 ml seawater with sediment concentrations up to 4 g/liter.

Results:

Clam larval growth was retarded in 1.0-2.0 g/liter silt with no normal development in 3.0 or 4.0 g/liter. Mortalities were >90% at 0.25 g/liter chalk and 0.5 g/liter in clay and Fullers earth. Clam larvae were able to survive up to 4.0 g/liter silt.

Cummins, J. M. 1973. Results of 48-hr embryo bioassay of Duwamish River bottom sediments. Final Rep. by U. S. Environmental Protection Agency, Region X Laboratory, Manchester, WA. 7 pp.

Pacific oyster (*Crassostrea gigas*) 48-hr larval survival/abnormality bioassay was used to test for toxicity of sediment cores collected from the Duwamish River in June 1973. Ten Duwamish samples plus Burley Lagoon sediment (control) and two seawater control samples (Burley Lagoon and Elliott Bay) were tested with oyster embryos at 20 °C, 29.5‰ salinity, pH 7.6-8.1. All samples were tested within 24 hours of collection.

Methods:

Sediment samples were prepared by mixing 100 g sediment with 500 ml Burley Lagoon seawater for 2 min and making serial dilutions to equal 0.01, 0.1, 1 and 10 g sediments (wet wt)/liter seawater. The sediments were then a final mix in the test bottles and allowed to settle prior to addition of the embryos (which may be strip-spawned due to failure of thermal spawning).

Results:

Most sediment samples exceeded 5% abnormal in the 1 and 10 g/liter concentrations including the Burley Lagoon control sediment. Control seawater = 0.91% abnormal and 100% survival (assigned, not actually counted relative to time zero). Survivals were generally reduced in all sediment samples and generally poor in the 1 and 10 g/liter concentrations. Elliott Bay seawater showed no significant responses.

Cummins, J. M. 1974. Oyster embryo bioassay of seawater and sediments from the Duwamish River, Elliott Bay and Clam Bay, Washington. Final Rpt. by the U. S. Environmental Protection Agency, Region X Laboratory, Manchester, WA. 8 pp.

The Pacific oyster (*Crassostrea gigas*) 48-hr larval survival/abnormality assay was used to test sediments from the Duwamish River, Elliott Bay and Clam Bay (control) in August 1974.

Methods:

Sediments were tested at concentrations of 0.01, 0.1, 1 and 10 g/liter in polyethylene beakers. Interstitial waters were prepared by centrifuging sediments and filtering the supernatant through 0.8 μ m filters and adding 0.1 to 100 ml/liter to seawater. Elutriates were also tested by preparing according to COE (1974) Elutriate Test guidelines (mixing, settling, centrifuging and filtering) and testing at concentrations of 0.2 to 200 ml/liter seawater. All tests were 48-hr exposures at 18-22 °C, salinity 26.0-29.5 ‰ and pH 7.8-8.1. Testing was initiated within 24 hours of sample collection. Oysters were strip-spawned due to lack of response to thermal stimulation.

Results:

Clam Bay control seawater = 8.02% abnormal and assigned a 48-hr survival of 100% (no time zero counts were made in the controls). The control abnormal exceeded the criterion for control abnormal of $\leq 5\%$. Almost all test samples had % abnormalities $<$ the control with the highest abnormal (11.9%) in one of the Duwamish samples. Mortality seemed to be a more sensitive indicator of toxicity in this test with all test samples registering $<100\%$ relative survival (embryos possibly lost in the sediments??). Of major interest was the fact that the lowest survivals were in the Clam Bay (control) sediments with survivals as low as 12.8% in the 10 g/liter concentration.

Davis, H. C. and H. Hidu. 1969. Effects of turbidity-producing substances in sea water on eggs and larvae of three genera on bivalve mollusks. *The Veliger* 11(4):316-323.

The authors exposed American (*Crassostrea virginica*) and European (*Ostrea edulis*) oyster embryos and larvae to silt, clay, Fullers earth and silicon dioxide particulates in 7-12 day growth/survival tests. Embryos were exposed in bottles rotated at 8 RPM at 23-25 °C, salinity = 26-27.5 ‰, and fed algae every 2nd day.

Results:

As little as 0.188 g/liter silt caused significant decreases in normal development. Decreased development also took place in 3 g/liter kaolin (clay) and in 4 g/liter Fullers earth. The smallest

particles (<5 μm) of silicon dioxide had the greatest effect on survival and growth. European oyster larvae were less affected than American oyster larvae. Low concentrations of suspended material apparently protected against low concentrations of natural toxins (possibly bacterial) or toxicants in seawater and produced better growth results.

Dinnel, P. A., S. C. Crumley and Q. J. Stober. 1979. Sand dollar (*Dendraster excentricus*) sperm and embryo bioassay of Puget Sound receiving water samples. Final Rpt. for Washington State Shellfish Laboratory, Brinnon, WA by Fish. Res. Institute, University of Washington, Seattle, WA. FRI-UW-7912. 19 pp.

The University of Washington and the Washington Shellfish Lab conducted parallel testing on 10 Puget Sound water samples collected by WDF in July 1979. The University ran sand dollar sperm/fertilization and embryo (abnormality only) assays. WDF ran Pacific oyster embryo (mortality and abnormality) assays.

Methods:

Sand dollar sperm assay: 25-ml subsamples, 30 and 60 min sperm exposures, salinity = 26.2-30.2 ‰, pH = 7.4-8.2, sperm:egg ratio = 1000:1, 4 replicates.

Sand dollar embryo assay: 3 replicates, 100 ml samples, 72-hr exposures.

Oyster embryo assay: Woelke (1972) method, 20 °C, 48-hr exposures.

Results:

Sand dollar sperm assay showed reduced fertilization success in one sample only (from Everett) where % fertilization was reduced to 47.8 and 40.8 for 30 and 60-min exposures (vs. 90% for the controls).

Sand dollar embryo assay showed significant abnormality only in the Everett sample, although 2 control samples showed "hits" for unknown reasons. Mortality was not measured.

Oyster embryo assay showed increased abnormals in the Everett sample and decreased survival in the Twanoh and Budd Inlet samples. Control mortality mean = 21% and mean abnormal = 3.3 %.

Thus, all 3 tests agreed that the one Everett sample was toxic. Factors affecting oyster survival in the 2 other samples are unknown.

Dinnel, P. A. and R. M. Kocan. 1988. Puget Sound Estuary Program sediment bioassay comparison test: Results of the sand dollar (*Dendraster excentricus*) embryo bioassays. Final Rpt. for Battelle Laboratories and the U. S. Environmental Protection Agency, Region X, Seattle, WA. by Marine Biological Consultants of Washington. MBCW-8802. 18 pp.

The authors conducted sand dollar embryo bioassays of sediments collected from Commencement Bay, Elliott Bay and Eagle Harbor as part of a sediment bioassay comparison test using a multitude of bioassay techniques (see PTI (1989) for a full analysis of all tests combined).

For the sand dollar assay: Used 20 g sediment/liter of seawater in glass beakers with 48-hr exposures. Sediment samples were mixed and allowed to settle at least 1 hr before embryo inoculation. Temperature = 15-16 °C, salinity = 29 ‰, pH = 6.25-7.96, DO = 4.8-7.7 mg/liter. Subsamples of the embryos were collected at 18 hrs for anaphase aberration assessments. Survival in the seawater controls of the first run = 65%. Hence, the test was rerun a second time where seawater control survival = 85% and abnormal = 3%. Test endpoints = 18 hr anaphase aberration and # of mitoses/embryo; 48-hr mortality; and 48-hr abnormality.

Results:

Survival range in the test sediments	= 0-111.2%
Abnormal range in test sediments	= 2.9-100%
# mitoses range in test sediments	= 0-11.0 (sw control = 10.6)
Abnormal mitoses in test sediments	= 0.9-6.2 (sw control = 1.1)

Meador, J. P., B. D. Ross and P. A. Dinnel. 1989. Sensitivity of sand dollar (*Dendraster excentricus*) early development to elutriates of Puget Sound sediments. Manuscript accepted for publication in Marine Environ Res.

Sand dollar embryos were exposed to dilutions (1, 2.5, 5, 10 and 16.6% v/v) of sediment elutriates of a stock slurry of 2:1 (= a 33.3% slurry). Sediments for Test 1 = frozen at 0 °C and for Test 2 = twice frozen.

Elutriates of sediments were prepared by mixing sediments 2:1 with seawater in 4-liter polyethylene containers for 30 min, allowed to settle for 1+ hr and filtered through 1.2 µm GF/C glass microfiber filters.

Results:

Only embryo abnormality was assessed. Generally good correlations were found between abnormality and cluster groups of sediments based on magnitude of chemical contamination. Test 2 showed a good dose-responsiveness. The twice-frozen sediments were substantially more toxic (less binding of toxicants??).

Phelps, H. L. and K. Warner. 1988. Development of an estuarine sediment solid-phase bioassay using oyster (*Crassostrea gigas*) larval mortality and metamorphosis. Final Rpt. to U. S. Fish and Wildlife Service, Annapolis, MD by University of the District of Columbia, Washington, D. C. 30 pp.

This testing used 4-day exposures of pediveliger larvae (several weeks old) in 2.75 ml glass wells with 1 ml of sediment and 1 ml seawater. Assessed mortality and metamorphosis. It proved difficult to assess larval mortality, but vital staining helped. 20-40 larvae were exposed in each well and were treated with epinephrine to induce metamorphosis.

Results:

Metamorphosis in the controls averaged only about 37%. Competency between the various oyster batches was rather variable. Copper was used as a positive control but poorly quantified. Storage of the control and Baltimore Harbor test sediments caused major changes in the toxicity responses.

Phelps, H. L. and K. A. Warner. In press. Estuarine sediment bioassay using oyster (*Crassostrea gigas*) larvae. *Bull. Environ. Contam. and Toxicol.* In press.

The authors explored the use of an oyster metamorphosis assay for testing the toxicity of natural sediments, aged sediments, frozen vs. unfrozen sediments and copper-spiked sediments. One set of tests was also conducted with the east coast oyster, *Crassostrea virginica*.

Methods:

Eyed pediveliger *C. gigas* were obtained via Federal Express from Coast Oyster Company, Washington State, and maintained in aerated artificial seawater in 2.7 liter glass jars, with antibiotics, and fed *Monocrysis* and *Isocrysis*, until used.

Control sediments were collected from west Chesapeake Bay and contaminated sediments came from Baltimore Harbor. Some of each sediment type was stored at 4° and 0 °C (frozen) for various lengths of time to age.

Bioassays were conducted by exposing 30 larvae for 96 hours to 1.0 ml sediment in 1.0 ml of artificial seawater in 2.75 ml tissue culture plate wells at 21 °C. Test endpoints = percent survival and percent metamorphosis. To induce metamorphosis, 0.0001 M epinephrine was added to the test dilution water at the beginning of the test.

Results:

Mortality of *C. gigas* in artificial seawater alone = 2.8% and for the control sediment = 6.4%. However, metamorphosis success was highly variable due to variable degrees of competency of the larvae. No metamorphosis took place at salinities <23.5 %, but survival was good down to 5.2 %.

Mortalities in control sediment increased from 6% to 48% following 115 days of storage at 4 °C and corresponded to decreases in pH from 8.0 to 6.0. Baltimore Harbor sediment was 100% toxic at day 0 but decreased to only 12% (for frozen sediments) to 44% (for 4° sediments) mortality at day 7. No metamorphosis took place in the Baltimore Harbor sediment.

The LD50 for copper-sorbed sediment = 313 mg/liter, although copper in sediment and pore water was not measured. Also, there was no metamorphosis in the copper-enriched sediment.

One set of tests with *C. virginica* showed 10% control mortality but only 3.5% metamorphosis. More work needs to be done with this species.

Schink, T. D., R. E. Westley and C. E. Woelke. 1974. Pacific oyster embryo bioassays of bottom sediments from Washington waters. Final Rpt. to the U. S. Army Corps of Engineers (Contract No. DAWC 67-72-C-0097) and the U. S. Environmental Protection Agency by the Washington Department of Fisheries, Olympia, WA. 24 pp.

Sediment samples from Grays Harbor, Duwamish River, Olympia Harbor, Henderson Inlet, Oro Bay, Bellingham Bay, Eld Inlet, Liberty Bay and Point Whitney Lagoon were tested for toxicity using a modified Pacific oyster larval abnormality test.

Methods:

Bioassay method = 29-hr exposures of embryos seeded in the test samples at the 19-hr old shell-less veliger stage at 20.0 ± 0.5 °C. Total bioassay volume = 800 ml in plastic bottles rotated at 4 RPM to suspend the sediments. Sediment concentrations tested ranged from 0.05 to 179 g/liter (wet wt) with about 5-10 concentrations tested per sediment sample. Many physical/chemical parameters were also measured. DOs in the bioassay cultures ranged from a low of 0.6 to 9.4 mg/liter. Dilution water was from Pt. Whitney. Salinities and pHs not given.

Results:

Seawater control abnormalities ranged from 0.3 to 2.6%. Test sediment abnormalities ranged from 0.2 to 100% with responses being generally dose-related. Some sediments from Grays Harbor and the Duwamish required <0.2 g/liter to elicit significant abnormal increases while sediments from other areas required >30 g/liter.

Relative toxicity was Grays Harbor > Duwamish & Bellingham Bay > Pt. Whitney Lagoon > Henderson Inlet > Eld Inlet > Oro Bay > Liberty Bay > Budd Inlet.

An attempt was made to relate toxicities to the measured physical/chemical parameters. Highest correlations (step-wise multiple regressions) were with total sulfides, zinc, sediment particle size, BOD and total phosphorus. Thus, sulfides (esp. H₂S) apparently have a significant effect on development, while there may also have been some effect due to physical abrasion caused by rotation of the samples.

Warner, K. A. 1988. Assessment and development of a pediveliger oyster larvae bioassay for determining the toxicity of contaminated marine and estuarine sediment. Summer Student Fellow Rpt., Woods Hole Ocean. Institution, MA. 30 pp.

This study compared *Crassostrea gigas* and *C. virginica* mortality and metamorphosis assay with the sensitivity of amphipod (*Ampelisca abdita*) mortality in natural sediments. Oyster assays = 4-day exposures of pediveliger larvae to sediments in 2.75 ml wells (1 ml sediment with 1 ml seawater). Temp. = 24 °C, no feeding.

Results:

There was generally good agreement for the sediment responses for both the larval mortality and metamorphosis and the amphipod mortality. Larval competency was an important interactive variable which varied between batches. It was also not easy to distinguish between metamorphosed and unmetamorphosed larvae. "The results from the oyster bioassay suggest that larval recruitment would be poor in those areas shown to inhibit metamorphosis."

WATER COLUMN

Calabrese, A., R. S. Collier, D. A. Nelson and J. R. MacInnes. 1973. The toxicity of heavy metals to embryos of the American oyster *Crassostrea virginica*. Marine Biology 18:162-166.

The authors exposed developing oyster embryos to 11 heavy metals in synthetic seawater to determine toxicity in terms of LC0s, LC50s and LC100s.

Methods:

Oysters were spawned into artificial seawater with thermal stimulation and sperm addition to the females. Exposures to various concentrations of metal salt ions took place in artificial seawater at 26 ± 1 °C, 25 ‰ salinity, pH = 7.0-8.5, exposure times of 42 to 48 hours with 15,000 - 17,000 embryos per 1 liter beaker (polypropylene and 2 replicates/7-12 concentrations. The test endpoint = "number of embryos that survived and developed into larvae."

Results (LC50s in mg/liter of the added metal ion—not metal salt:

Metal	LC50
Mercuric chloride	0.0056
Silver nitrate	0.0058
Cupric chloride	0.103
Zinc chloride	0.31
Nickel chloride	1.18
Lead nitrate*	2.45
Cadmium chloride	3.80
Sodium arsenite	7.50
Chromium chloride*	10.3
Manganese chloride	16.0
Aluminum chloride*	Not calculated, LC0 = 7.5

* = Precipitates present in the exposure beakers.

Calabrese, A., J. R. MacInnes, D. A. Nelson and J. E. Miller. 1977. Survival and growth of bivalve larvae under heavy-metal stresses. Marine Biology 41:179-184.

This study exposed larvae of American oysters, *Crassostrea virginica*, and hard clam, *Mercenaria mercenaria*, to mercury, silver, copper, nickel and zinc to determine the effects on survival and growth.

Methods:

C. virginica: Adult oysters were spawned by thermal stimulation. 10,000 to 12,000 48-hour larvae were exposed to seawater/metal solutions in 1 liter polypropylene beakers containing 1 μ m-filtered natural seawater at 24 ± 2 ‰ salinity at 25 ± 1 °C for 12 days. Larvae were fed two species of algae. Three replicates per concentration were used. Test endpoints = survival and growth. Tests were terminated by screening larvae with 36 μ m mesh Nytex screen. LC50s were calculated via linear regression analysis.

M. mercenaria: Same as for the oyster larvae except that exposure times = 8-10 days.

Results:

LC5s, LC50s and percent growth at the LC50 concentrations as μ g/liter of metal ion only were as follows:

Toxicant	LC5	LC50	% Growth at the LC50
<i>C. virginica</i>			
Mercuric chloride	3.3	12.0	49.1
Silver nitrate	14.2	25.0	67.1
Cupric chloride	10.0	32.8	67.7
Nickle chloride	30.0	1,200.0	45.2
<i>M. mercenaria</i>			
Mercuric chloride	4.0	14.7	68.7
Silver nitrate	18.6	32.4	66.2
Cupric chloride	4.9	16.4	51.7
Nickle chloride	1,100.0	5,700.0	0.0
Zinc chloride	50.0	195.4	61.6

Growth was generally unaffected at the LC5 concentrations.

Cardwell, R. D., C. E. Woelke, M. I. Carr and E. W. Sanborn. 1976a. Toxicity of marine waters near Everett and Port Angeles, Washington, to larval Pacific oysters in 1975. Final Report by Washington Department of Fisheries, Point Whitney Shellfish Laboratory, Brinnon, WA. 88 pp.

This study investigated the toxicity of marine waters in and around Everett/Port Gardner and Port Angeles in 1975. This was a continuation of a toxicity testing program initiated in 1972. The primary test organism was larvae of the Pacific oyster, *Crassostrea gigas*, but some comparative tests of paper mill effluents were also conducted with rainbow trout and oyster larvae.

Methods:

Oyster larvae tests were conducted 18-19 August and 25 August 1975. Field samples of marine waters were collected by Van Dorn bottle from the surface and at various depths and flown to the Pt. Whitney lab for testing. Grab and composite effluent samples were also collected from the various paper mills in Everett and Port Angeles. The test protocol was that of Woelke (1972) modified to exclude the terminal larvae filtration step. Test samples having salinities <20 ‰ were adjusted upwards with high salinity water produced by freezing seawater to concentrate the salts.

Results:

For the seawater controls, oyster larval survival ranged from 74.9% to 96.9% and abnormalities ranged from 0.6% to 4.0%. EC50s for DDS (used as a reference toxicant) ranged from 0.78 to 1.03 mg/liter and LC50s ranged from 0.88 to 1.08 mg/liter.

In general, water quality in the Everett area steadily improved from 1972 to 1975. The improved water quality "corresponded largely to improvements in pulp mill wastewater treatment."

For the Port Angeles area, water quality remained poor from 1972 to 1974, but improved substantially in 1975 when the ITT Rayonier pulp mill instituted incineration of its sulfite waste liquor.

Toxicity of Scott Paper Mill (Everett) effluents ranged from 0.029% to 1.58% for abnormality (EC50) and from 0.96% to 23.8% for survival (LC50). Toxicity of ITT Rayonier Mill (Port Angeles) effluents ranged from 0.015% to 0.38% for abnormality and from 1.25% to 17.5% for survival. The oyster larvae were 58-476 times more sensitive to the ITT Rayonier effluents than rainbow trout based on oyster larval EC50s. The oyster LC50s were 0-7 times lower than rainbow trout.

There were few statistically significant correlations between oyster responses and the Pearl-Benson Index (PBI) for sulfite waste liquors.

Cardwell, R. D., C. E. Woelke, M. I. Carr and E. W. Sanborn. 1976b. Toxicity of marine waters near Everett and Port Angeles, Washington to larval Pacific oysters 1972 through 1975. Chapter IV In: Ecological Baseline and Monitoring Study for Port Gardner and Adjacent Waters: A Summary Report for the Years 1972 Through 1975. Report No. DOE 76-20, Washington Department of Ecology, Olympia, WA.

The authors used Pacific oyster (*Crassostrea gigas*) larval bioassays to measure marine water quality in and around Port Gardner and Port Angeles from 1972-1975. They also conducted bioassays of several pulp mill effluents.

Methods:

The authors used the Woelke (1972) standard oyster embryo bioassay method without the termination filtration step. 48-hr exposures at 20 °C with the endpoints of survival and abnormality. Bioassays were conducted on the same day as sample collection. Various depths sampled. Samples with salinities <20‰ were adjusted upward with freeze-concentrated natural seawater brines.

Results:

Seawater control responses for four tests: Survival = 74.9, 96.9, 87.6, 87.7%; Abnormalities = 1.0, 0.6, 4.0, 1.2%. Oysters were conditioned in the lab for 59-69 days prior to spawning. Results of the field testing for Everett area showed decreasing areal extent of marine water toxicities through time, probably due to increased pulp mill effluent treatment requirements. Marine water toxicity in the Port Angeles area remained essentially the same from 1972-1975, but pulp mill effluent treatment not initiated until 1975.

The abnormality index was a much more sensitive indicator of pulp mill effluents (EC50 range = 0.015 to 0.38% effluent) than mortality (LC50 range = 1.25-17.5% effluent). Both measures were more sensitive than fingerling rainbow trout (LC50 range = 7-36% effluent). Larval abnormality correlated with the Pearl-Benson Index (PBI = one measure of sulfite waste liquor concentrations) better than mortality. However, the relationships were highly variable.

Cardwell, R. D., C. E. Woelke, M. I. Carr and E. W. Sanborn. 1977. Evaluation of water quality of Puget Sound and Hood Canal in 1976. NOAA Tech. Memo. ERL MESA-21, Marine Ec. systems Analysis Program, Boulder, CO. 36 pp.

The Woelke (1972) method was used to bioassay Puget Sound and Hood Canal water samples with 48-hr exposures of Pacific oyster (*Crassostrea gigas*) embryos.

Methods:

Test conditions: 48-hr test at 20 °C in 950 ml samples. Control water came from 18 m depth in Dabob Bay. Samples were tested same day as collection, one batch in July 1976 and the other in Sept 1976. Test endpoints = larval abnormality and mortality (corrected for the 48-hr control responses—no time zero counts). Some ancillary tests were also conducted.

Results:

Seawater Control responses:

Test #1 = 12.0% mortality and 1.9% abnormal
 Test #2 = 25.9% mortality and 1.6% abnormal

For the test samples: Increased abnormality was only seen in the Eld and Henderson Inlet samples and from the surface waters of Commencement Bay (with decreased salinities and increased PBIs). Higher mortalities were observed for many samples from South Puget Sound and South Hood Canal and at the heads of some of the inlets. Mortality in some cases was >99%.

Ancillary tests and data analyses were highly suggestive of mortality being associated with densities of the dinoflagellate, *Ceratium fusus*. Other test manipulations of sunlight, artificial light, filtration and heating had little effect on the test results except that filtration at 8 μm mitigated mortality. However, no conclusive results to these tests. Tetracycline (for bacteria control) also proved toxic in one set of tests. Bacterial metabolites were also considered as one possible source of "natural toxicity."

Cardwell, R. D., C. E. Woelke, M. I. Carr and E. W. Sanborn. 1977. Appraisal of a reference toxicant for estimating the quality of oyster larvae. *Bull. Environ. Contam. Toxicol.* 18(6):719-725.

The authors exposed batches of Pacific oyster (*Crassostrea gigas*) embryos from 20 different spawning pairs to the reference toxicant Dodecyl Sodium Sulfate (DSS) to assess quality of embryos.

Results:

Survival was >70% and abnormal > 90% in all 20 tests. Abnormality and mortality were poorly correlated in the controls. The relationship between abnormality and mortality of control larvae to sensitivity to DSS was quite weak. The only significant relationship was between the time required to induce spawning in the females and sensitivity of progeny to DSS (the longer the time to spawning, the more sensitive to DSS).

For all 20 tests:

Mean control abnormal	=	2.1 \pm 2.2% (SD)
Mean control survival	=	92.4 \pm 10.9%
Mean LC50 for DSS	=	0.91 mg/liter

$$\text{Mean EC50 for DSS} = 0.84 \text{ mg/liter}$$

Cardwell, R. D. and C. E. Woelke. 1979. Marine water quality compendium for Washington State. Volume I. Introduction. Final Rpt., Grant No. R805032010, for the U. S. Environmental Protection Agency, Corvallis, OR by Washington Department of Fisheries, Olympia, WA. 75 pp.

The primary objective of this project was to develop computer programs and software requisite for managing and utilizing oyster and clam larval bioassays of Washington State marine waters conducted from about 1961-1976. Volume I contains a discussion of methodology, basics of the bioassay procedures, sources of variability and data analyses. Basically, it provides a summary overview of the State testing program since 1961 with references to a variety of "special studies" conducted to elucidate toxicity patterns in problem areas (especially around pulp and paper mills) and as related to "natural" larval mortality probably caused by phytoplankton and/or bacterial metabolites.

This report contains the details of computer programs for managing and analyzing bioassay data (developed by the UW). Gives detailed discussions of response criteria and statistical procedures used by the State. This volume also provides a partial analysis of bioassay data from West Central Puget Sound and Willapa Bay as examples of the software capabilities. It identifies Sequim and Discovery Bays as areas which typically caused high larval mortalities. Willapa Bay generally tested clean.

Cardwell, R. D., C. E. Woelke, M. I. Carr and E. W. Sanborn. 1979. Toxic substance and water quality effects on larval marine organisms. Tech. Rpt. No. 45, Washington Department of Fisheries, Olympia, WA. 71 pp.

This is basically a synthesis report of past work conducted by the WDF Brinnon Shellfish Lab on oyster/clam larval bioassays. It discusses biomonitoring and bioassays in general and especially as applied to receiving water quality in Puget Sound. Topics covered in the report include refinement of the bioassay methodology (e.g., deletion of larval screening at test termination, use of better pipettes, T_0 counts from the control beakers for later mortality counts, addition of a larger volume of spawn), use of a reference toxicant, tests on the relative sensitivity between oysters and clams, data analysis, and results of tests of various chemicals, effluents and receiving waters.

General Conclusions:

- 1) The test is improved (less variability, less bias) by use of better pipettes, no termination filtration step and by the addition of T_0 counts from the control beakers (vs. counts from just the stock beaker).
- 2) The sensitivity of the larvae to a reference toxicant (dodecyl sodium sulfate) was not related to the magnitudes of control mortality/abnormality.
- 3) Details the results of Summer tests in 1975 and 1976 showing that the range of control survival = 74.9 to 100% and control abnormal = 0.0 to 10.3% (20 tests).
- 4) Age of the water and pH can be factors affecting toxicity.
- 5) Gives toxicity data on the following: Sediments, sodium sulfide, H_2S , tannic acid, salinity, pH, ammonia-N, DO, antibiotics, petroleum, linear alkylate sulfonate surfactants, cadmium, methoxyclor, various pulp mill effluents and receiving water samples.

6) Oyster and clam larvae are generally of comparable sensitivity to toxicants.

Cardwell, R. D., S. J. Olsen, M. I. Carr and E. W. Sanborn. 1980. Biotic, water quality and hydrologic characteristics of Skyline Marina in 1978 Tech. Rpt. No. 54, Washington Department of Fisheries, Olympia, WA. 103 pp.

The authors conducted Pacific oyster (*Crassostrea gigas*) larval bioassays of three water samples from Skyline Marina in August 1978. Water samples were also tested from outside the marina and from three areas in the general vicinity of the marina. Exposure time = 48 hr at 20 °C, Woelke (1972) protocol.

Results:

Laboratory seawater controls = 13.04% mortality and abnormality = 1.94%. All test samples showed negligible toxicity with mortality ranges of 0 to 8.81% and abnormality of 0.26 to 3.97%.

Bioaccumulation tests for metals were also conducted using adult oysters.

Carr, M., C. Woelke, W. Hoffman and E. Sanborn. 1974. Bivalve embryo bioassays of marine waters and industrial waste samples from the Sandy Point to Point Whitehorn area in Puget Sound, Washington. Final Rpt. By the Washington Department of Fisheries, Olympia, WA. 18 pp.

WDF Brinnon Lab conducted 48-hr Pacific oyster embryo (*Crassostrea gigas*) bioassays of receiving waters collected from the Cherry Point area of North Puget Sound on 5 Sept. 1973. Water samples were collected from the vicinity of Intalco, ARCO, and Mobil facilities and from Lummi Bay. The bioassays were conducted at 20 ± 0.5 °C, control salinity = 30.2 ‰, pH = 7.64. Some testing of effluents was also conducted.

Results:

Control abnormal = 0.28%, carry-along control abnormal = 0.77%. Control survival set to 100% (no T_0 counts in the controls). Several field water samples were found to be highly toxic (93-99% abnormal) and a few more found to be of low to moderate toxicity. Toxic areas were greatly reduced as compared to similar surveys conducted in 1971 and 1972. The reduced field toxicity was probably due to the new installation of effluent treatment facilities in the previous year. Most effluent samples tested were of little or no toxicity at 1:5 dilutions.

Cipollaro, M., G. Corsale, A. Esposito, E. Rarucci, N. Staiano, G. G. Giordano and G. Pagano. 1986. Sublethal pH decrease may cause genetic damage to eukaryotic cell: A study on sea urchins and *Salmonella typhimurium*. *Teratogenesis, Carcinogenesis and Mutagenesis* 6:275-287.

The authors exposed sea urchin embryos to low pH caused by additions of HCl, H₂SO₄ and H₃PO₄. Sperm were also exposed to assess degrees of sperm inactivation.

Methods:

Test species = *Paracentrotus lividus* and *Sphaerechinus granularis*. Test temp. = 20 °C in natural seawater. Exposure times for cytogenetic analyses = 5 hours.

Results:

Adverse effects to development were observed at pHs of ≤7.5. Sperm inactivation was progressively delayed by decreasing pHs. H₃PO₄ was the most toxic to sperm.

Davis, H. C. 1961. Effects of some pesticides on eggs and larvae of oysters (*Crassostrea virginica*) and clams (*Venus mercenaria*). Comm. Fish. Rev. 23(12):8-23.

The author exposed oyster and clam larvae to various pesticides, oils, solvents, antibiotics, bactericides and disinfectants. The tests were conducted over a period of several years.

Methods:

Larval exposures were conducted in 1 liter cultures in 1,500 ml glass beakers with feeding every day and duplicate test concentrations. UV-treated seawater was used for dilution and control water. Test endpoints = % of larvae developed to the straight-hinge stage in 48 hrs and growth following 12 to 14 days of exposure. Test water and toxicant solutions were replaced at 2-day intervals.

Results:

The results were presented as bar graphs showing % development or % growth at each test concentration. The table below presents approximations of the EC50s (in mg/liter) based on interpolations from the bar graphs. NT = not tested.

Compound	Development	Growth	Clam or Oyster
Phenol	10-100	~10	C
Roccal	~0.2	0.10-0.20	C
Nemagon	>10	0.25-0.50	C
Dowicide A	~10	0.5-1.0	C
Dowicide G	<0.25	<0.25	C
Nabam	<0.50	<0.50	C
Sulmet	NT	~100	C
Chloramphenicol	NT	10-100	C
Delrad	NT	~0.05	C
Allyl Alcohol	~1.0	<0.25	C
Ortho Dichlorobenzene	>10	>10	C
Trichlorobenzene	~10	>5	C
Acetone	>100	>250	C
Monuron	>5	>5	C
Diuron	1-5	>5	C
Fenuron	>5	>5	C
Neburon	<2.4	<2.4	C

Compound	Development	Growth	Clam or Oyster
Sevin	2.5-5.0	1-2.5	C
Lindane	>10	>10	C
Toxaphene	~1.0	<0.25	C
Guthion	0.5-1.0	1-5	C
Aldrin	>10	<0.25	C
Dicapthon	2-10	2-5	C
Compound 3514	<1.0	<1.0	C
Sevin	1-5	NT	O
Lindane	5-10	NT	O
Guthion	0.5-1.0	>1.0	O
Compound 3514	<1.0	<1.0	O
Dieldrin	1-2.5	1-2.5	O
Endrin	1-10	~2.5	O

Davis, H. C. and H. Hidu. 1969. Effects of pesticides on embryonic development of clams and oysters and on survival and growth of the larvae. Fish. Bull. 67(2):393-404.

The authors exposed American oyster, *Crassostrea virginica*, and hard clam, *Mercenaria mercenaria*, embryos and larvae to 52 compounds including insecticides, herbicides, solvents, bactericides, fungicides and algicides.

Methods:

Embryos and larvae were exposed for 48 hrs (to straight-hinge stage for development success) or 10-12 days (for growth) to toxicant/seawater solutions in Pyrex culture vessels at 24 ± 1 °C. Seawater and test solutions were replaced at 2 day intervals. Larvae were fed daily with live flagellates.

Results:

EC50s interpolated from the development and growth data are presented in tabular form below (all concentrations are in mg/liter):

Compound	Oyster		Clam	
	48-hr EC50	14-day EC50	48-hr EC50	14-day EC50
Insecticides				
Aldrin			>10.0	0.41
Co-Ral	0.11	>1.0	9.12	5.21
DDT		0.034		
Dicapthon			3.34	5.74
Dieldrin	0.64	>10.0		
Dipterex		1.0		
Di-Syston	5.86	3.67	5.28	1.39
Endrin	0.79	>10.0		
Guthion	0.82		0.86	0.86

Compound	Oyster		Clam	
	48-hr EC50	14-day EC50	48-hr EC50	14-day EC50
Lindane	9.1		>10.0	>10.0
Malathion	9.07	2.66		
N-3452	<0.5	<0.5		
N-3514	<1.0	<1.0	<1.0	<1.0
Sevin	3.0	3.0	3.82	>2.50
TEPP	>10.0	>10.0		
Toxaphene			1.12	<0.25
Herbicides				
Amitrol	733.7	255.4		
Amitrol-T	>10.0	>10.0		
2-4 D ester	8.0	0.74		
2-4 D salt	20.44	64.29		
Diuron			2.53	>5.0
EMID	16.82	30.00		
Endothal	28.22	48.08	51.02	12.50
Fenuron			>10.0	>5.0
MCPA	15.62	31.30		
Monuron			>5.0	>5.0
Neburon			<2.4	<2.4
Silvex	5.90	0.71		
Nematocide				
Nemagon			10.0	0.78
Solvents				
Acetone	>100.0		>100.0	>100.0
Allyl Alcohol			1.03	<0.25
Ortho Dichlorobenzene			>100.0	>100.0
Trichlorobenzene	3.13		>10.0	>10.0
Bactericides, fungicides algicides & misc.				
Chloramphenicol			74.29	50.0
Delrad	0.31			0.072
Dowicide A			>10.0	0.75
Dowicide G			<0.25	<0.25
Griseofulvin			<0.25	<1.0
PVP-Iodine			17.10	34.94
Nabam	<0.5		<0.5	1.75
Nitrofurazone			>100.0	>100.0
Omazene	0.78	0.34	0.081	0.378
Pentachlorophenol	<0.25	0.071		
PCP acetate	<0.25	<0.025		
Phenol	58.25		52.63	55.0
Phygon	0.014	0.041	0.014	1.75

Compound	Oyster		Clam	
	48-hr EC50	14-day EC50	48-hr EC50	14-day EC50
Roccal			0.19	0.14
Rosin Amine D	<0.25	<0.025		
Sulmet, tinted			>100.0	>100.0
Sulmet, untinted	>600.0	>600.0	>1,000	>1000
TCC			0.032	0.037
TCP	0.60	>1.0		

deAngelis, E. and G. G. Giordano. 1974. Sea urchin egg development under the action of benzo(a)pyrene and 7, 12-dimethylbenz(a)anthracene. *Cancer Research* 34:1275-1280.

The authors exposed sea urchin eggs to the above noted chemicals during development and prior to fertilization to define effects of these human carcinogens to development.

Methods:

Test species = *Paracentrotus lividus*, test temp. = 18-20 °C with natural seawater. BP & DMBA were dissolved in <0.1% DMSO. Development exposure times = 6 to 24 hours. Egg and/or sperm exposures prior to fertilization = 2 hours.

Results:

Effects of BP and DMBA were different. Both compounds showed toxicity in the range of about 10^{-4} to 10^{-5} molar.

Eyster, L. S. and M. P. Morse. 1984. Development of the surf clam (*Spisula solidissima*) following exposure of gametes, embryos and larvae to silver. *Arch. Environ. Contam. and Toxicol.* 13:641-646.

Toxicity tests determined the sensitivity of clam fertilization, embryos and larvae to silver added to seawater for various exposure times.

Methods:

Testing utilized static cultures in 1 liter beakers with silver nitrate added to UV-treated, 0.22 µm-filtered seawater at 20 °C, 30‰ salinity and pH ~8.0. Silver concentrations tested ranged from 0.6 to 64 µg/liter, nominal. Testing used 2-4 replicates of each concentration and tests were repeated 2-7 times. Gametes from 2 males and females were pooled for each test. Spawning was generally accomplished by slitting the gonads. Exposure times were up to 48 hrs with egg densities of 30 eggs/ml.

Results:

Average control 48-hr development to the D-shaped veliger = 96%. Eggs pretreated for 45 minutes prior to fertilization produced abnormal development, especially at Ag concentrations >9.5

$\mu\text{g/liter}$. Sperm pretreatments of 45 min produced normal fertilization at 16 $\mu\text{g/liter}$, but abnormal embryos often resulted. Short (2-hour) exposures of 24-hr old embryos to 0-16 $\mu\text{g/liter}$ Ag produced no abnormal development. Continuous exposures for 48 hours produced significant abnormal development at Ag concentrations $\geq 9.5 \mu\text{g/liter}$. High mortalities were observed in 16 and 32 $\mu\text{g/liter}$ Ag.

Glickstein, N. 1978. Acute toxicity of mercury and selenium to *Crassostrea gigas* embryos and *Cancer magister* larvae. *Marine Biology* 49:113-117.

The author exposed Pacific oyster embryos and Dungeness crab first zoea to mercury and/or selenium to measure the toxicities of each alone and the two toxicants in combination.

Methods:

Test dilution water = filtered and UV-treated natural seawater. Tests were conducted at the Calif. Fish & Game Marine laboratory at Granite Canyon. Stock solutions of toxicants were prepared in HCl-acidified distilled water and the metal concentrations verified by AAS. Seawater parameters during testing were: pH = 8.1 ± 0.2 ; DO = 6.5-8.0 mg/liter; salinity = $33.8 \pm 0.1 \%$; temperature = $20 \pm 1^\circ\text{C}$ or $15 \pm 1^\circ\text{C}$ for oysters and crab, respectively. It was not given if the metal concentrations are reported as concentrations of the metal ions or of the parent compound.

For oyster larvae, 6,000 to 7,000 fertilized eggs were incubated for 48 hrs in 250 ml of test solution in polypropylene Tripour beakers. Embryos were sieved at termination through 35 μm mesh Nytex screen. Test endpoint = normal development to the "D"-shaped veliger stage.

For crab zoea, tests started within 60 hours of egg hatching. Larvae were fed brine shrimp, *Artemia salina*, before testing and at 48 hours. Zoea were exposed for 48 to 96 hrs in 250 ml of test solution, five zoea/beaker. Test endpoint = death.

Results:

The LC50s (crab zoea) and EC50s (oyster embryos) in $\mu\text{g/liter}$ were (NT = not tested):

Toxicant	Time (hours)	Oyster Embryo	Crab Zoa
Hg(NO ₃) ₂	48	5.5	NT
HgCl ₂	48	5.7	21.1
HgCl ₂	96	NT	6.6
Na ₂ SeO ₃	48	>10,000	NT
SeO ₂	48	>10,000	5,090
SeO ₂	96	NT	1,040

In combination, high concentrations of selenium ($\geq 5,000 \mu\text{g/liter}$) enhanced the toxicity of mercury, whereas moderate levels (100 - 1,000 $\mu\text{g/liter}$) of Se tended to decrease the toxicity of Hg.

Based on a radioisotope tracer study, the concentration of dissolved Hg decreased by 57% of the original dose in seawater in 40 hours while Se remained stable.

Hagstrom, B. E. and S. Lonning. 1973. The sea urchin egg as a testing object in toxicology. *Acta Pharmacologica et Toxicologica* 32:(Supp. 1):1-49.

This is one of the earliest papers which propose that sea urchin fertilization and early development can be used as sensitive biological measures of chemical toxicity including lethal, developmental, cytological and cytogenetic endpoints.

Methods:

The authors conducted fertilization rate tests and developmental abnormality assessments following 3-hour exposures of developing embryos (embryos were exposed for 3-hour periods at various stages of development). Test temp. = 18-20 °C, test species = *Paracentrotus lividus* and *Psammechinus microtuberculatus*.

Results:

This paper reports the effects of the following chemicals on fertilization rate and development: Chloramphenicol, nicotine, chlorpromazine, imipramine and thalidomide. The results also include cytological studies of the exposed embryos.

Heslinga, G. A. 1976. Effects of copper on the coral-reef echinoid *Echinometra mathaei*. *Marine Biology* 35:155-160.

The authors exposed gametes, embryos and adults of the sea urchin *Echinometra mathaei* to various concentrations of this metal.

Methods:

Copper as CuCl_2 was added to 0.45 μm -filtered seawater of salinity = 32.6 to 35 ‰, pH = 8.2-8.5 and temperature = 28 ± 0.5 °C. Concentrations of copper tested ranged from 0.02 to 0.67 mg/liter (nominal).

Fertilization assays were conducted by simultaneous exposures of sperm and eggs to 20 ml of copper solutions for 10 minutes prior to fixation. Embryos were exposed at early cleavage for 110 minutes and assessed for successful division to the 8 cell stage. Larvae were exposed, starting at the blastula stage, to Cu for 24, 48, 72 and 96 hrs and survival, normal development and length of the pluteus arm skeleton assessed. Adults (20-30 mm and 45-50 mm diameter groups) were exposed to Cu for 96 hrs and survival assessed.

Results:

Fertilization success was reduced by 25% at 0.05 mg/liter Cu and nonexistent at 0.67 mg/liter. Cleavage to the 8 cell stage was a less sensitive indicator of toxicity with 90% cleavage at 0.22 mg/liter and 2.6% cleavage at 0.67 mg/liter. Inhibition of skeletal development was first observed in 0.02 mg/liter. 0.11 mg/liter Cu was lethal to all larvae in 96 hrs. Adults of both size ranges were equally sensitive to Cu with 96-hr LC50s of 0.30 mg/liter and 48-hr LC50s of ~0.54 mg/liter.

Kobayashi, N. 1971a. Fertilized sea urchin eggs as an indicatory material for marine pollution bioassay, preliminary experiments. *Publ. Seto Marine Biol. Lab.* 28 (6):379-406.

This is the primary early publication by Kobayashi which provides the basic format for conducting sea urchin embryo bioassays. He gives a supporting philosophy for the conduct of embryo bioassays, discusses appropriate species, sea urchin stages of development, provides a testing protocol ("Manual for Bioassay") and the results of field water testing and testing of individual chemicals.

Appropriate sea urchin species/spawning times/temperatures:

<i>Hemicentrotus pulcherrimus</i>	Jan-March	14-16 °C
<i>Anthocidaris crassispina</i>	May-August	20-28
<i>Pseudocentrotus depressus</i>	Oct-Nov	18-23
<i>Temnopleurus torenumaticus</i>	July-August	?

Results:

Used several species of urchins to test field water samples from areas suspected to be free of pollution plus areas known to be polluted (Osaka Harbor) and found a range of effects in the embryo assays (development to the gastrula stage only). Testing also investigated the use of artificial sea salts and boiled seawater to adjust sample salinities. Both had adverse effects.

Results of single chemical tests with *Anthocidaris crassispina* at 28 °C:

Toxicant	Effect Range (ppm)	Toxicant	Effect Range
Phenyl mercuric acetate	0.0018-0.009	Mercuric chloride	0.046-0.023
Cupric sulfate	0.1-0.05	Zinc chloride	0.13-0.065
Nickle chloride	1.2-0.6	Cadmium chloride	1.6-0.8
Lead acetate	2.2-1.1	Chromium chloride	8.4-4.2
Manganese chloride	13.2-6.6	Cobalt acetate	170-17
Potassium cyanide	0.25-0.125	Ammonium chloride	3.3-0.33
Arsenic pentoxide	4.2-2.1	Formaldehyde	3.7-0.37
Alkyl benzene	31-15.5	Phenol	31-15.5
Sodium sulfate	156-78	Sodium fluoride	220-110
Boric acid	900-450		

Kobayashi, N. 1971b. Bioassay data for marine pollution using sea urchin eggs, 1970. *Publ. Seto Marine Biol. Lab.* 18:421-424.

This paper reports results of bioassays of 1970 field-collected seawaters from the area around the Seto Marine Laboratory, Japan. Bioassays evaluated fertilization success (3 min) and larval development to the gastrula stage (10-32 hour exposures) using *Anthocidaris crassispina* and *Pseudocentrotus depressus*.

Results of these tests indicated little if any toxicity in the field samples.

Kobayashi, N. 1972. Bioassay data for marine pollution using sea urchin eggs, 1971. Publ. Seto Marine Biol. Lab. 19:439-444.

The author used larval development to gastrula (12-36-hr exposures) and fertilization success (3-min) of three species of sea urchins (*Hemicentrotus pulcherrimus*, *Anthocidaris crassispina* and *Pseudocentrotus depressus*) to test seawater quality in the vicinity of the Seto Marine Laboratory, Japan.

Results of these tests indicated no toxicity in the field samples.

Kobayashi, N. 1973. Studies on the effects of some agents on fertilized sea urchin eggs, as part of the basis for marine pollution bioassay, I. Publ. Seto Marine Biol. Lab. 21 (2):109-114.

The author investigated the effects of tannic acid, Kaolin, pH and elevated temperatures on the fertilization success and larval development to gastrula of *Anthocidaris crassispina*. Testing was conducted in 1971 at temperatures of 26-28 °C.

Results:

Tannic acid: Minimum effective concentration = 6.3 ppm
Maximum effective concentration = 3.1 ppm

Kaolin: Minimum effective concentration = 500 ppm
Maximum effective concentration = 250 ppm

pH: Fertilization affected at ≤ 7.4 and ≥ 9.4
Gastrulation affected at ≤ 7.4 and ≥ 9.0

Temperature: Fertilization affected at ≥ 33 °C
Gastrulation affected at ≥ 31 °C

Kobayashi, N. 1974a. Bioassay data for marine pollution using sea urchin eggs, 1972 and 1973. Publ. Seto Marine Biol. Lab. 21:411-432.

The author conducted sea urchin fertilization (3-min) and larval development (to gastrula) bioassays of field-collected seawater samples from around the Seto Marine Laboratory. Species used were: *Hemicentrotus pulcherrimus*, *Anthocidaris crassispina* and *Pseudocentrotus depressus*. Incubation temperatures = 13-28 °C depending on species/season.

Results showed that most samples were "clean" with only a few samples producing slight effects on fertilization and development.

Kobayashi, N. 1974b. Marine pollution bioassay by sea urchin eggs, an attempt to enhance accuracy. Publ. Seto Marine Biol. Lab. 21 (5/6):377-391.

The author investigated the effect of sea urchin egg aging (prior to fertilization) on fertilization success and development to gastrula with and without toxicants for two species: *Hemicentrotus pulcherrimus* and *Anthocidaris crassispina* at 26-28 °C.

Results:

Hemicentrotus eggs could age up to 6 hrs without significant effects on fertilization and development, but *Anthocidaris* eggs could only tolerate 3 hrs, possibly due to higher temperatures for this species. Aging of eggs for 3 hrs indicated an increase in sensitivity to a few toxicants, but generally this did not increase sensitivity of the assay.

One contaminated sediment sample from an industrial area was tested and an elutriate of this sample substantially affected fertilization and development. Kobayashi also proposed a slightly modified "Manual for Bioassay" and a new "Ranking II" chart for assessing degrees of effects of toxicants on fertilization and development.

Kobayashi, N. 1977. Preliminary experiments with sea urchin pluteus and metamorphosis in marine pollution bioassay. Publ. Seto Marine Biol. Lab. 24 (1/3):9-21.

This work investigated the relative sensitivity of the pluteus larval stage (as compared to gastrulation) of the sea urchin *Anthocidaris crassispina* and the sand dollar *Peronella japonica* and metamorphosis of *Peronella*, all at 26 °C. Toxic chemicals and field-collected water samples were tested with both species.

Results (lowest effective concentrations in mg/liter):

Chemical	<i>Anthocidaris</i>		<i>Peronella</i>	
	Gastrula	Pluteus	Gastrula	Pluteus
HgCl ₂	0.03	0.03	0.015	0.015
CuSO ₄ · 5H ₂ O	0.25	0.06	0.03	0.015
ZnCl ₂	0.06	0.06	0.015	0.015
CdCl ₂ · 2-1/2 H ₂ O	3.0	3.0	1.2	1.2
K ₂ Cr ₂ O ₇	25.0	6.0	1.2	0.6
ABS	1.5	1.5	0.6	0.3
NH ₄ Cl	3.0	3.0	1.2	0.6
As ₂ O ₅	3.0	3.0	0.8	0.2

Peronella was more sensitive than *Anthocidaris* in all cases. The pluteus stage was almost always a more sensitive indicator of toxicity than prior stages or 3-min. fertilization success tests. These same patterns of sensitivity were also demonstrated with field-collected seawater samples.

Metamorphosis in *Peronella* is exceptionally short (3-4 days) and may be useful as a supplemental assay. It is especially important to note that metamorphosis is more sensitive to toxicants than earlier developmental stages.

Kobayashi, N. 1980. Comparative sensitivity of various developmental stages of sea urchins to some chemicals. Marine Biology 58:163-171.

The author compared sensitivities of various stages of development through metamorphosis for the Japanese sand dollar *Peronella japonica* at 26 °C and the Australian sea urchin *Heliocidaris erythrogramma* at 24 °C. Data are presented for copper, NH₃ and ABS for these species and comparative data from previous testing with other Japanese species are also presented.

Results:

Only copper was tested with both species; sensitivity to copper was essentially the same. Threshold concentration of copper for *H. erythrogramma* was estimated to be about 0.001 mg/liter over ambient (0.003 mg/liter) seawater concentrations. The relative sensitivity of different developmental endpoints = Sperm (pre-exposure) > metamorphosis and gastrula > fertilization > blastula and pluteus > cleavage > young adults.

Kobayashi, N. 1981. Comparative toxicity of various chemicals, oil extracts and oil dispersant extracts to Canadian and Japanese sea urchin eggs. *Publ. Seto Marine Biol. Lab.* 26 (1/3):123-133.

The author determined the relative sensitivities of developing embryo stages of *Strongylocentrotus droebachiensis*, *Anthocidaris crassispina*, *Hemicentrotus pulcherrimus* and *Pseudocentrotus depressus* to various metals and chemicals including oil and an oil dispersant.

Results:

Species	Estimated Threshold Concentration (mg/liter) for Cleavage					Source
	Cu	Zn	Cd	ABS	NH ₃	
<i>S. droebachiensis</i>	0.02	0.02	0.5	1.0	1.0	This paper
<i>P. depressus</i>	0.03	0.03	1.0	1.0	2.0	This paper
<i>H. pulcherrimus</i>	0.05	0.05	2.0	1.0	1.0	This paper
	0.006	0.07	0.1			Published lit.
<i>A. crassispina</i>	0.06	0.06	1.5	1.5	3.0	This paper
	0.03	0.03			3.2	Published lit.
<i>Arbacia punctulata</i>	0.001	0.002	0.5			Published lit.

For oil and/or dispersant:

Species	Thresholds for Delayed Development (mg/liter)		
	Bunker C oil	BP 1100X Dispersant	Oil/Dispersant
<i>S. droebachiensis</i>	5.0	20,000	2.0 + 10,000
<i>H. pulcherrimus</i>	5.0	20,000	Not tested
<i>A. crassispina</i>	10.0	20,000	5.0 + 10,000

The oil/dispersant mix was more toxic than oil or dispersant alone. Generally, there was only a small difference in sensitivities between species. However, there may be a small trend as follows: *Arbacia punctulata* > *S. droebachiensis* > *P. depressus* > *H. pulcherrimus* > *A. crassispina*. There are probably some differences due to differences in testing protocols, temperatures, etc.

Kobayashi, N. 1985a. Marine pollution bioassay by sea urchin eggs, an attempt to enhance accuracy, II. *Publ. Seto Marine Biol. Lab.* 30 (4/6):213-226.

The author investigated the relative sensitivity of pretreatment of eggs (3 & 6 hrs) and/or sperm (5 min) on sensitivity of ensuing development of *Anthocidaris crassispina* and *Hemicentrotus pulcherrimus* to copper, zinc, ABS and ammonium chloride at temperatures of 19-26 °C.

Results:

The trend in sensitivity to toxicants relative to exposure condition = pre-treated eggs + pre-treated sperm > normal eggs + pre-treated sperm > pre-treated eggs + normal sperm > normal eggs + normal sperm. This pattern was also evident for testing of natural seawaters. These results stimulated a new proposal for a modified "Manual for Bioassay" and a new ranking scheme (III) for ranking toxicant effects.

Kobayashi, N. 1985b. Studies on the effects of some agents on fertilized sea urchin eggs, as part of the bases for marine pollution bioassay, II. The Science and Engineering Review of Doshisha University 26(1):1-7.

The author quantified the effects of 12 chemicals on the development of two species of sea urchins (*Hemicentrotus pulcherrimus* and *Anthocidaris crassispina*) using the "Manual of Bioassay" previously developed in 1971 and modified in 1984. The test temperature = 19-20 °C.

Results:

Chemical	Effects Threshold (mg/liter)
	<i>Hemicentrotus</i>
AlCl ₃ · 6H ₂ O	5
FeCl ₃ · 6H ₂ O	20
PCP	0.1
PCB (KC400)	0.5
Phthalate DBP	1
Phthalate DOP	10
Sumithion	10
Cement	100
NaNO ₂	50
NaNO ₃	500
	<i>Anthocidaris</i>
ABS	5
LAS	2

Kobayashi, N. 1986. The inhibitory action of heavy metals and its recovery by EDTA on fertilized sea urchin eggs: Preliminary report. The Science and Engineering Review of Doshisha University 26(4):49-55.

The author used development of sea urchin (*Anthocidaris crassispina*) embryos at 25 °C to test toxicities of 7 metals with and without EDTA (a chelator) added. Also, the author tested metal refinery effluent with and without EDTA.

Results:

Chemical	Lowest Effect Concentrations in mg/liter	
	Metal Alone	Metal + EDTA
CuSO ₄	0.2	~0.5
ZnCl ₂	0.2	~0.5
K ₂ Cr ₂ O ₇	20	<20
Pb(CH ₃ COO) ₂	20	<20
NiCl ₂	2	<2
FeCl ₃	10	<10
AlCl ₃	5	<5

The toxicity of metal refinery effluent was greatly reduced by the addition of EDTA.

Kobayashi, N., H. Nogami and K. Doi. 1972. Marine pollution bioassay by using sea urchin eggs in the Inland Sea of Japan (the Seto-Naikai). *Publ. Seto Marine Biol. Lab.* 19(6):359-381.

The authors collected about 200 water samples (surface and bottom) from throughout the Inland Sea of Japan in 1971 for testing with sea urchin (*Anthocidaris crassispina* at 28 °C) fertilization and development to the gastrula stage. Test waters were frozen at -20 °C prior to testing. Information on COD, metals in bottom muds and benthic infaunal data were also collected.

Results:

Fertilization and development were successful in many samples but severely retarded in others. Bioassay results were poorly correlated with COD but some slight relationship was seen with metal concentrations and benthic infaunal biomass/composition. The authors present a table suggesting tentative rankings of inhibitory effects of water pollution on fertilization and development.

Kobayashi, N. and K. Fujinaga. 1976. Synergism of inhibiting actions of heavy metals upon the fertilization and development of sea urchin eggs. *The Science and Engineering Review of Doshisha University* 17 (1):54-69.

The authors tested the toxicity of four metals (Cu, Zn, Ni, Cd) to sea urchin fertilization and development using two species of urchins (*Hemicentrotus pulcherrimus* at 20 °C and *Anthocidaris crassispina* at 28 °C) and paired combinations of those metals. This article is in Japanese with English tables and figures.

Results:

Hemicentrotus was more sensitive to metals than *Anthocidaris*. Cu + Zn were strongly synergistic and Cu + Cd and Zn + Cd moderately synergistic. Additive toxicity was observed for Cu + Ni, Zn + Ni and Ni + Cd. Concentration and effect levels for single metals and combined metals are presented in multiple tables.

Martin, M., K. E. Osborn, P. Billig and N. Glickstein. 1981. Toxicities of ten metals to *Crassostrea gigas* and *Mytilus edulis* embryos and *Cancer magister* larvae. *Marine Poll. Bull.* 12(9):305-308.

The authors conducted embryo/larval assays of ten metals with the above noted test animals. Stock solutions of the metals were verified with AAS. The oyster and mussel embryo assays were 48-hour exposure tests at 20 ± 1 °C and 17 ± 1 °C, respectively. Crab larvae assays were 96-hr exposures at 15 ± 1 °C (probably).

Results (EC50 or LC50 as µg/liter of the metal ion):

Metal	Species		
	<i>C. gigas</i> (48-hr EC50)	<i>M. edulis</i> (48-hr EC50)	<i>C. magister</i> (96-hr LC50)
CuSO ₄	5.3	5.8	49
HgCl ₂	6.7	5.8	8.2
AgNO ₃	22	14	55
ZnSO ₄	119	175	456
AsO ₅	326	>3,000	232
NiSO ₄ · 6H ₂ O	349	891	4,360
CdCl ₂	611	1,200	247
Pb(NO ₃) ₂	758	476	575
K ₂ CrO ₇	4,538	4,469	3,440
SeO ₂	>10,000	>10,000	>10,000

Okubo, K. and T. Okubo. 1962. Study on the bio-assay method for the evaluation of water pollution - II. Use of fertilized eggs of sea urchins and bivalves. *Bull. Tokai Regional Fish. Res. Lab.* 32:131-140.

The authors tested the sensitivity of sea urchin, bivalve and crustacean larvae to a variety of substances added to seawater. The article is in Japanese with tables and synopsis in English. The crustacean values are probably comparisons from previous work. Contains many pictures of normal and abnormal development.

Results:

Substance (mg/liter)	Lowest Effective Concentrations			
	<i>Anthocidaris</i> (27 °C)	<i>Hemicentrotus</i> (11-16 °C)	<i>Mytilus</i> (13-17 °C)	<i>Crassostrea</i> (27 °C)
Cu	0.1	0.032	0.1	0.1
Hg	0.032	0.032	0.032	0.032
Fe	10	-----	32	-----
Mn	32	-----	32	-----
Zn	0.32	-----	1.0	3.2

Substance (mg/liter)	Lowest Effective Concentrations			
	<i>Anthocidaris</i> (27 °C)	<i>Hemicentrotus</i> (11-16 °C)	<i>Mytilus</i> (13-17 °C)	<i>Crassostrea</i> (27 °C)
Cr	10	<1.0	10	-----
CN	0.1	0.1	0.1	0.1
CrO ₄	10	10	32	32
NH ₄	10	10	32	32
I ₂	-----	3.2	-----	-----
Na ₂ SO ₃	3200	32	<3200	3200
Chrome alum	100	-----	100	320
Picric acid	100	32	320	32
Tannic acid	-----	10	10	-----
Phenol	-----	-----	100	-----
Parathion	-----	1.0	1.0	-----
Uranine	-----	32	3.2	-----
Rodamin B	-----	32	10	-----
Alcohol (% vol)	1.0	-----	3.2	-----
Acetone (% vol)	1.0	3.2	3.2	-----
Chloroform (% vol)	-----	10	10	-----

Substance (mg/liter)	Crustaceans			
	<i>Artemia</i> (20 °C)	<u>24-hour TL₅₀</u>	<i>Sesarma</i> (zoea) (27 °C)	<u>Not Affected</u>
			Adult <i>Balanus</i> (27 °C)	Nauplii (27 °C)
Cu (CuSO ₄)	0.68-1.04	5.2	3.2	3.2
Cu (CH ₃ COOCu)	2.5	6.0	-----	-----
Hg	21-50	0.06	0.32	0.1
Fe	34-62	-----	-----	-----
Mn	1570-2880	500	100	100
Zn	160-275	6.2	32	3.2
Cr	40	56	3.2	-----
CN	0.8-1.2	1.8	3.2	3.2
CrO ₄	70	200	-----	-----
NH ₄	400-700	140-180	32-320	100-320
I ₂	2.3	-----	-----	-----
Na ₂ SO ₃	140-240	1000	320	1000
Chrome alum	140-220	180	-----	-----
Picric acid	12-64	160	32	100
Tannic acid	500	360	-----	-----

Substance (mg/liter)	<u>24-hour TL_m</u>		<u>Not Affected</u>	
	<i>Artemia</i> (20 °C)	<i>Sesarma</i> (zoea) (27 °C)	Adult <i>Balanus</i> (27 °C)	Nauplii (27 °C)
Phenol	200-300	-----	-----	-----
Parathion	3.6	0.0037	-----	-----
Uranine	100-300	-----	-----	-----
Rodamin B	180	-----	-----	-----
Alcohol (% vol)	2.4	3.7	1.0	3.2
Acetone (% vol)	1.3-1.9	4.5	<10	-----
Chloroform (% vol)	5.8-10	6.0	-----	-----

Oshida, P. S. 1977. Toxicity of a chlorinated benzene to sea urchin embryos. Pp. 187-192 *In: Coastal Water Research Annual Report 1977*, South. Calif. Coast. Water Res. Proj., El Segundo, CA. 253 pp.

The author exposed developing purple sea urchin (*Strongylocentrotus purpuratus*) embryos to ortho-dichlorobenzene (O-DCB) to determine it's effects on development.

Methods:

Fertilized sea urchin eggs (2.3-2.7 million/liter) were added to 2 liter jars to which O-DCB was added via the aeration line (800 ml/min). Approximate O-DCB concentration was 21 mg/liter. Exposures were up to 52 hrs (subsamples taken at various times) at 17 °C in natural 0.45 µm-filtered seawater.

Results:

For controls: 98% reached blastula in 5 hrs, 94% were at gastrula in 28 hrs and 89% were at late prism/early pluteus at 52 hrs. For the O-DCB exposed embryos, development was the same as the controls up to 24 hrs. However, development was abnormal (high % exogastrula) past 24 hrs. None developed to the prism/pluteus stage in 52 hrs.

Oshida, P. S., T. K Goochey and A. J. Mearns. 1981. Effects of municipal wastewater on fertilization, survival, and development of the sea urchin, *Strongylocentrotus purpuratus*. Pp. 389-402 *In: Biological Monitoring of Marine Pollutants*. Academic Press, Inc.

The authors used purple sea urchin gametes and developing embryos to measure the toxicity of effluents of several Southern California sewage treatment plants.

Methods:

Gamete assays used 30 min pre-exposures of the eggs, 15 min pre-exposures of the sperm and a further 15 min (total 1 hr) co-exposure of sperm and eggs during fertilization. The test endpoint was fertilization success.

Embryo assays were conducted by adding 31,500 eggs to 900 ml of 3 μ m-filtered seawater/sewage dilutions and incubating at 12 °C for 48 hrs with stirring at 60 RPM with aeration. Test endpoint = normal development to gastrula.

Results:

Generally, reduced fertilization success paralleled reductions in normal development. Embryo development was a more sensitive indicator of toxicity than freshwater fish tests by 5-10 times. The range of effluent that reduced fertilization success = 0.14% (for sludge) to 20%. The range that affected normal development was 0.1% to 20%.

Pagano, G., A. Esposito, G. G. Giordano and B. E. Hagstrom. 1978. Embryotoxic and teratogenic effects of styrene derivatives on sea urchin development. *Scand. J. Work Environ. & Health* 4 (Suppl. 2):136-141.

Styrene and some of its derivatives were tested for toxicity and mutagenicity using a sea urchin test system at the Naples, Italy Zoological Station.

Methods:

Two species of sea urchins were used for testing: *Paracentrotus lividus* and *Psammechinus microtuberculatus*. Three types of exposures were used: 1) Embryo exposures (various types) during development to pluteus at ~45 hrs; 2) Pretreatment of eggs for 5 min followed by washing; and 3) Sperm pretreatment for 2 min prior to fertilization of the eggs. All test concentrations were reported as nominal—the actual dissolved concentrations were probably lower.

Results:

Effects were noted in the range of 10^{-3} to 10^{-5} molar for the various styrene compounds. Effects included reduced fertilization success, abnormal cleavage and later development, retarded development and cytolysis. It was especially noted that many effects were not evident until the later stages of development; hence, they were probably related to damage of the genome. The authors concluded that this test system, especially sperm pretreatment, is suited for detection of direct acting mutagens.

Pagano, G., A. Esposito and G. G. Giordano. 1982. Fertilization and larval development in sea urchins following exposure of gametes and embryos to cadmium. *Arch. Environ. Contam. Toxicol.* 11:47-55.

The "Sea Urchin Test System" of Hagstrom and Lonning was used to expose sperm, eggs and developing embryos to cadmium.

Methods:

Sea urchin species used = *Paracentrotus lividus*, *Psammechinus microtuberculatus* and *Sphaerechinus granularis*. Sperm were pretreated for 2 min prior to fertilization. Eggs were pretreated for 5-10 min prior to fertilization. Exposures during development to pluteus were also conducted.

Results:

10^{-3} M Cd^{2+} = No differentiation of the skeleton. 10^{-5} to 10^{-4} M Cd^{2+} = Skeletal injuries. Exposures at cleavage were not sensitive. Sperm/fertilization stimulation was observed at 10^{-8} M Cd^{2+} . No genotoxicity was observed (no mitotic aberrations).

Pagano, G., A. Esposito, P. Bove, M. deAngelis, A. Rota and G. G. Giordano.
1983. The effects of hexavalent and trivalent chromium on fertilization and development in sea urchins. *Environ. Res.* 30:442-452.

Sea urchin embryos and sperm were exposed to chromium to assess the effects on development success and fertilization rates.

Methods:

Test species = *Paracentrotus lividus* and *Sphaerechinus granularis*. Tests were conducted in filtered natural seawater, probably at 18-20 °C, and pH = 7.8-8.0. Sperm exposure times = 2-10 min. Eggs were pretreated up to 60 min prior to fertilization.

Results:

Embryo development was adversely affected (poor gut and skeleton development) by chromate (Cr^{6+}) at concentrations as low as 5×10^{-5} M. Pre-exposures of sperm to CrO_4^{2-} at 10^{-4} to 10^{-2} M resulted in abnormal larvae. Mitotic activity was also affected by CrO_4^{2-} . Cr^{3+} adversely affected motility and hatchability.

Pagano, G., M. Cipollaro, G. Corsale, A. Esposito, G. G. Giordano, E. Ragucci and N. M. Trieff. 1988a. Comparative toxicities of benzene, chlorobenzene and dichlorobenzene to sea urchin embryos and sperm. *Bull. Environ. Contam. Toxicol.* 40:481-488.

The toxicities of benzene compounds to sea urchin fertilization, development and mitotic activities are described in this paper.

Methods:

Test sea urchin = *Paracentrotus lividus*. Tests were conducted with filtered natural seawater at 20 °C, pH = 8.2-8.4 and salinity = 37.6 to 37.8 g/liter. Development exposure times = 48 hours. Sperm exposure times = 5 to 90 min (used pooled sperm from 4 males and eggs from a single female). Test endpoints = 1) sperm inactivation, 2) % development defects, 3) # of mitoses/embryo, 4) % interphase embryos, 5) metaphase/anaphase ratio, 6) % total mitotic aberrations and 7) % anaphase aberrations.

Results:

The order of toxicity of these compounds varied depending on whether sperm or eggs were treated. General effects levels were between 10^{-4} and 10^{-6} molar. Criteria for ranking toxicities of chemicals should be based on multiple tests, even when a single species is used.

Pagano, G., M. Cipollaro, G. Corsale, A. Esposito, A. Mineo, E. Ragucci, G. G. Giordano, N. Kobayashi and N. M. Trieff. 1988b. Effects of sodium azide on sea urchin embryos and gametes. *Teratogenesis, Carcinogenesis and Mutagenesis* 8:363-376.

Sea urchin embryos, eggs and sperm were exposed to sodium azide (SA) to assess the effects on development, genotoxicity and fertilization rates. Effects of pH were also investigated as a co-toxicant.

Methods:

Test sea urchin = *Paracentrotus lividus*. Eggs, sperm and embryos were exposed to SA in filtered natural seawater at 20 °C at varying pHs. Embryos were exposed for 8 or 48 hrs. Sperm were exposed for 10 to 120 min periods and eggs for 10 min.

Results:

48-hr embryo exposures produced adverse effects to skeletal differentiation at 10^{-4} to 10^{-3} molar SA. There were no cytogenetic abnormalities at these concentrations. Fertilization rates following sperm exposures were normal up to 10^{-2} M SA, but were affected by reduced pHs.

Tracy, H. B., R. A. Lee, C. E. Woelke and G. Sanborn. 1969. Relative toxicities and dispersing evaluations of eleven oil-dispersing products. *J. Water Poll. Control Fed.* 41(12):2062-2069.

The authors used coho salmon, steelhead trout and oyster embryo assays to determine toxicities of 11 oil dispersants in both freshwater and seawater. They also used *in situ* exposures of animals in cages in Hood Canal, WA.

Results:

No mortalities were observed for salmonid exposures to oil and/or dispersants in the *in situ* exposures. 96-hr mean tolerance limits (TL_m) were calculated for each dispersant for fingerling steelhead. TL_m s ranged from 3.2 to 65.0 mg/liter. Temperature = 16-18 °C, static test in 10-liter aquaria. Toxic concentrations for oyster embryo abnormality ranged from 0.001 to 40.0 mg/liter at 20 °C for 48-hr exposures. No embryo mortality data given.

Trieff, N. M., M Cipollaro, G. Corsale, A. Esposito, E. Ragucci, G. G. Giordano, S. V. N. Ramanujam, D. R. Livingstone and G. Pagano. 1988. Aroclor 1254 toxicity in sea urchin embryos and gametes. *Exp. Oncol.* 7:57-64.

The authors exposed sea urchin (*Sphaerechinus granularis*) embryos and gametes to the polychlorinated biphenyl (PCB) Aroclor 1254 to determine it's toxicity to fertilization and embryonic development.

Methods:

Embryos and gametes were exposed to nominal concentrations of PCB ranging from 10^{-6} M to 10^{-4} M (0.326 to 32.6 mg/liter). DMSO was used as a solvent to aid exposures in seawater.

Results:

Developmental toxicity was observed only if unfertilized eggs or hatched embryos were exposed. No effects were induced in cleaving (pre-hatch) embryos. Detectable concentrations of PCB were not taken up by the embryos. Spermotoxic effects were observed at PCB concentrations of 10^{-5} M, but 10^{-6} M caused enhanced fertilization capacity. Embryos from PCB-exposed sperm exhibited developmental defects, but no cytogenetic abnormalities, following sperm pretreatments at 10^{-6} to 10^{-5} M PCB.

Vashchenko, M. A. 1980. The effect of water soluble hydrocarbon fraction of diesel fuel on the development of gametes and quality of offspring of the sea urchin *Strongylocentrotus nudus*. Soviet J. Marine Biology (translation of Biologiya Morya) 6(4):236-242.

This study exposed sea urchin adults to "Brand L" light diesel fuel (30 ± 5 mg/liter) for 30-45 days during the period of gametogenesis. Condition of the developing gonads was monitored histologically and the quality of post-exposure embryonic development assessed with and without additional exposures to diesel fuel.

Methods:

Adult sea urchins were exposed to either clean seawater (controls) or seawater/diesel fuel in aerated aquaria with 2 liters seawater/animal, water was changed every third day and the animals were fed *Ulva* and *Laminaria*. Temperature was raised from 5-9 °C to 17 °C during the exposures to thermally induce gametogenesis during the test. Following adult exposures for 30 to 45 days, gametes and embryos were exposed to varying gradations of oil (0, 15, 30 and 60 mg/liter) in four combinations of control and oil-exposed gametes (e.g., control sperm X oiled eggs, etc.).

Results:

The adult exposures did not produce any signs of effects on gametogenesis, either grossly or histologically. However, fertilization and normal development was affected when gametes of oil-exposed urchins were used. In all cases, gametes from non-oil exposed adult urchins yielded good fertilization and development to pluteus. Normal development was reduced at gastrula and pluteus stages when gametes of one sex came from oil-exposed urchins and very greatly reduced when both gametes came from oiled parents. The degree of abnormality was also directly related to the degree of gamete/embryo exposure post-spawning.

Woelke, C. E. 1967. Measurement of water quality with the Pacific oyster embryo bioassay. Pp. 112 In: Water Quality Criteria, ASTM STP 416, Am. Soc. for Testing and Materials, Philadelphia, PA.

This was one of the first publications by Woelke on the use and methodology of a Pacific oyster (*Crassostrea gigas*) larval bioassay.

Major assumption stated in the paper:

"...that failure to develop to fully shelled larvae in 48 hours will break the life cycle of the Pacific oyster. I consider failure of the eggs to develop, or the proportion (per cent) of larvae developing in an abnormal manner to constitute a measure of the biological response to a particular stimulus."

This paper gives the basic steps for conducting oyster embryo bioassays and provides some data on the effects of various effluents and receiving water samples from around Port Angeles, WA.

Woelke, C. E. 1972. Development of a receiving water quality bioassay criterion based on the 48-hour Pacific oyster (*Crassostrea gigas*) embryo. Tech. Rpt. No. 9, Washington Department of Fisheries, Olympia, WA. 93 pp.

See this entry under Methodology for the results of 10 years of water quality and toxicant testing for the State of Washington. Also provides comparative test data from other sources for many toxicants and bioassay animals.

RELATED REFERENCES

Dinnel, P. A., G. G. Pagano and P. S. Oshida. 1988. A sea urchin test system for marine environmental monitoring. Pp. 611-619 *In: Echinoderm Biology*, R. D. Burke, P. V. Mladenov, P. Lambert and R. L. Parsley, eds. A. A. Balkema, Rotterdam.

This review paper describes the use of all life stages of sea urchins and sand dollars in marine toxicological testing including adult behavior and physiology, embryo survival, development and physiology, gamete tests, and mitotic tests.

Lannan, J. E. 1980. Broodstock management of *Crassostrea gigas*. I. Genetic and Environmental variation in survival in the larval rearing system. *Aquaculture* 21:323-336.

Larval survival of Pacific oysters in a hatchery system was highly variable and due to both genetic and non-genetic components. Specific mating combinations influenced larval survival. This influence results from stringent genetic regulation of the rate of gametogenesis. For a given rearing environment, maximum larval survival occurs in matings between individuals expressing the optimum stage of gonadal development. The rate and timing of gametogenesis are under genetic control. Pacific oysters are evolutionarily adapted to reproduce only during certain seasons. Precise manipulation of gametogenesis is necessary to insure optimally conditioned broodstock.

Lannan, J. E., A. Robinson and W. P. Breese. 1980. Broodstock management of *Crassostrea gigas*. II. Broodstock conditioning to maximize larval survival. *Aquaculture* 21:337-345.

There is an optimal conditioning interval for oyster gametogenesis during which the proportion of viable gametes is at a maximum. When spawning is outside the optimum window, spawning and fertilization may appear normal, but survival may be low. The degree of condi-

tioning required to reach the optimum window is dependent on the stage of development in the broodstock when conditioning is started, and reflects substantial seasonal variation, which is repeated on an annual cyclic basis.

Lee, G. F., J. M. Lopez and M. D. Piwoni. 1976. Evaluation of the factors influencing the results of the elutriate test for dredged material disposal criteria. Pp. 253-288 *In: Proceedings of the Speciality Conference on Dredging and its Environmental Effects*, P. A. Krenkel, J. Harrison and J. C. Burdick, III, eds. Am. Soc. Civil Engineers, New York, NY.

The authors looked at effects of various variables affecting sediment elutriate preparation including sediment/water ratios, shaking times, mode of agitation, settling times, sample sizes, storage times, etc. on concentrations of dissolved substances in the "standard elutriate test."

Shaking times, mode of agitation, settling times and sample sizes all had little or no effect on the concentrations of dissolved metals (except manganese). The sediment/water ratio, storage times, anoxic vs. oxic conditions and ionic strength did have minor effects on metals concentrations.

O'Connor, T. P. 1976. Investigation of the elutriate test. Pp. 299-318 *In: Proceedings of the Speciality Conference on Dredging and its Environmental Effects*, P. A. Krenkel, J. Harrison and J. C. Burdick, III, eds. Am. Soc. Civil Engineers, New York, NY.

Sediment elutriates were investigated for dissolved metal concentrations relative to various variables including pH, DO, mixing, open vs. closed system, etc. Elutriates were 1:4 sediment (Washington Navy Yard):seawater, salinity = 35 ‰.

Results:

With mixing, pH usually dropped to ~6.5 and DOs dropped rapidly. High zinc concentrations were most associated with drops of pH to ≤ 6.5 . The amount of shaking time and sediment: water ratios had some effect on metal concentrations. Changes in DO affected the state of iron. With increased DOs, ferric hydroxide precipitates formed. These in turn can scavenge zinc from solution. Reductions in DOs possibly due to sulfides. Dissolved zinc concentrations also affected by grain size. Clays provide more surface area for adsorption. Suggested for toxicity tests that the water be aerated and the pHs normalized.

Shuba, P. J., J. H. Carroll and H. E. Tatem. 1976. Bioassessment of the standard elutriate test. Pp. 645-672 *In: Proceedings of the Speciality Conference on Dredging and its Environmental Effects*, P. A. Krenkel, J. Harrison and J. C. Burdick, III, eds. Am. Soc. Civil Engineers, New York, NY.

This study used freshwater and marine algae to conduct bioassays of elutriates prepared via the "standard elutriate test." Elutriates were prepared with a 1:4 sediment:water ratio, 30-min mixing, 1-hr settling, centrifugation for 10 min and filtration at $0.45 \mu\text{m}$.

Freshwater algae = *Seleniastrum capricornutum*
 Marine algae = *Dunaliella tertiolecta*

Test sediments = Ashtabula Harbor, Lake Erie (freshwater)
Houston Ship Canal, Texas (seawater)

Results:

Algal growth inhibition occurred in elutriates from both sediment sources as compared to disposal site water without sediments. There was some stimulation of growth in some of the elutriate dilutions.

CHAPTER 4. POLYCHAETE (*NEANTHES ARENACEODENTATA*) BIOASSAYS

METHODOLOGY

Johns, D. M., T. C. Ginn and D. J. Reish. 1989a. Interim protocol for juvenile *Neanthes* bioassay. Final Report, PTI Contract C901-07 for the Washington Department of Ecology, Olympia, WA by PTI Environmental Services, Bellevue, WA. 14 pp. + appendices.

This document provides interim methodology for conducting bioassays of Puget Sound sediments using 20-day solid phase sediment exposures to *Neanthes* sp. with lethal and sublethal endpoints. This document also includes background information on past use of this species for toxicity testing, species sensitivity, ecological importance and use constraints. Appendices include: 1) *Neanthes* Protocol Workshop Meeting summary, 2) *Neanthes* bibliography and 3) past *Neanthes* test data.

Interim *Neanthes* protocol specifications:

- 1) Use cultured animals obtained from out-of-state. This species is not native to Puget Sound.
- 2) Worms should be 2-3 weeks post-emergence and in a rapid growth phase.
- 3) Sediment tests are run for 20 days with 1-liter beakers for the exposure chambers, 2 cm sediment, with aeration, static-renewal system (1/3 of the seawater replaced every 3 days), temperature = 20 ± 1 °C, salinity = 28 ± 1 ‰ and 40 mg of food added at start and every other day.
- 4) Tests are terminated at 20 days by screening with a 0.5 mm screen.
- 5) Test endpoints = survival, total biomass and average individual biomass.
- 6) Parameters monitored during the test = temperature, DO, salinity and pH.
- 7) Positive control test (e.g., cadmium chloride, 96-hr LC50 without sediments) should be run in parallel.

Johns, M. J. and T. C. Ginn. 1990. Development of a *Neanthes* sediment bioassay for use in Puget Sound. Final Report for the U. S. Environmental Protection Agency, Region X, Office of Puget Sound by PTI Environmental Services, Bellevue, WA. 62 pp. + appendices.

This report provides a recommended protocol for using *Neanthes* in a sublethal bioassay of sediments. The authors investigated factors which may affect the results of sediment assays using *Neanthes*. The factors tested were: number of organisms/beaker; amount of food ration; static vs. static renewal test system; exposure duration; salinity; and grain size.

Methods:

Natural sediments collected from West Beach, Whidbey Island (control), Carr Inlet (reference), Elliott Bay (contaminated) and Duckabush estuary (for a salinity gradient) were used as the test sediments. Sediment chemical measurements were made on Carr Inlet and Elliott Bay sediments for organics, metals and conventional. Sediments were stored at 4 °C under nitrogen until used (no storage times given).

Neanthes were obtained from Don Reish cultures. The basic protocol of Johns et al. (1989a) provided the basic format for the tests: 2-3 week old juveniles were exposed for 20 days to 2 cm sediment in 1 liter jars with 28 ‰ salinity seawater, a static renewal system and 40 mg of food (TetraMarin) every 2nd day. The test endpoints = survival, total biomass and average individual biomass. Experimental modifications to this basic protocol were:

- Worm densities were tested at 5, 10, 15 and 20 worms/jar
- Food ration was tested at 0, 20, 40, 60, and 80 mg/48 hrs
- Static vs. static renewal (1/3 seawater changed every 3rd day)
- Test exposure durations of 10, 15 and 20 days
- 96-hour salinity tolerance (water only) test at 10, 15, 20, 25 and 28 ‰, and 20-day interstitial salinity tolerance (with Duckabush sediments) at 15, 22, 25 and 30 ‰ (but 28 ‰ overlaying water salinity)
 - Sensitivity to varying silt/clay fractions
 - Sensitivity of *Neanthes* to cadmium chloride ($CdCl_2$) in water.

Results:

Worm density tests: Survival as a function of density was affected in Elliott Bay sediments but not in Carr Inlet sediments. Worm densities were also a factor in the biomass measurements. However, densities >5 worms/ jar did not increase the statistical power of the test; thus, use 5 worms/jar in future standard tests.

Food ration: Both survival and growth increased up to the highest food ration, but toxic effects of Elliott Bay sediments decreased with increases in food. Fungal growths were also observed at high food rations. Therefore, use 40 mg/48 hrs as a compromise feeding level.

Static vs. static renewal: No significant differences in survival or biomass observed between the two systems. However, a static system had higher ammonia levels while the static renewal system lost very little of the measured toxicants. Thus, use a static renewal system for future tests.

Test duration: Survival decreased slightly with exposure time and biomasses increased. However, statistical discrimination power was best with a 20 day exposure; thus, use 20 days.

Salinity: Mortalities were observed at <20 ‰ and the LC50 for salinity was 15 ‰ for 96-hr exposures to water. However, survivals were high at all interstitial salinities using the Duckabush sediments. Possible behavior effects and/or "dilution" of the interstitial salinity (by the overlaying 28 ‰ water) may be factors for this. Thus, use overlaying water salinity of 28 ‰ and use care when testing sediments with interstitial salinities of <20 ‰.

Grain size: *Neanthes* showed good survivals in all grain sizes tested.

Sensitivity to cadmium: The 96-hr LC50 (water only) = 22 mg/liter as $CdCl_2$. Use $CdCl_2$ as a reference toxicant for future tests.

Reish, D. J. 1980. Use of polychaetous annelids as test organisms for marine bioassay experiments. Pp. 140-154 In: Aquatic Invertebrate Bioassays, A. L. Buikema, Jr. and J. Cairns, Jr., eds. ASTM STP 715. Am. Soc. for Testing and Materials, Philadelphia, PA.

Since polychaetes constitute over 40% of the number of species and specimens in subtidal soft-bottom benthos, regardless of depth or latitude, then they are an obvious choice for bioassay tools.

Advantages of polychaetes:

1. Faunal importance
2. Ease of handling
3. Short life histories
4. Ease of culture and transport
5. Some large enough for body burden testing

Some disadvantages:

1. Lack of standardization of testing procedures
2. Lack of trained personnel
3. Many species of small size

Discusses specific protocols for using 3 species of polychaetes in bioassays and gives line drawings of each species. The 3 species are:

1. *Neanthes arenaceodentata*
2. *Capitella capitata*
3. *Ctenodrilus serratus*

Reish, D.J. 1985. The use of the polychaetous annelid *Neanthes arenaceodentata* as a laboratory experimental animal. *Tethys* 11(3-4):335-341.

A laboratory population of *Neanthes* was established by Reish at Long Beach State Univ. in 1964. These lab stocks should minimize natural variability. This worm species is known by three names:

Neanthes arenaceodentata (Moore, 1903)
N. acuminata (Ehlers, 1868)
N. caudata (delle Chiaje, 1828)

Full life cycle = 3-4 months at 20-22 °C. Oshida has shown that the effects of cadmium was identical on both lab and field-collected animals. Six labs conducted identical tests with silver and endosulfan and found low between-lab variability in worm sensitivities. The bioassay methodology for this species has been published in Standard Methods and other publications. This article gives a summary table of *Neanthes* sensitivities to physical factors, nutrients, metals, pesticides and oil (Table 1). Table 2 in the article gives *Neanthes* reproductive sensitivities to metals and oil. Favorable factors for use of this species as a bioassay tool = ease of culture &

transport, short life-cycle, small number of fairly large eggs, and isogenetic strain of 70-80 generations.

SEDIMENTS

Johns, D. M. and T. G. Ginn. 1989b. Test demonstration of a 10-day *Neanthes* acute toxicity bioassay. Final Report, PTI Contract # C843-06 for the U. S. Army Corps of Engineers, Seattle District by PTI Environmental Services, Bellevue, WA. 16 pp. + appendices.

The authors conducted 10-day acute bioassays of clean and contaminated Puget Sound sediments using *Neanthes* sp. in static and static-renewal test systems with variable numbers of worms per treatment.

Methods:

Neanthes were exposed for 10 days to 2-cm depths of sediments collected from Elliott Bay (contaminated), Carr Inlet (clean reference) and West Beach (control), Puget Sound, WA. Test temperature = 18.5-20 °C, salinity = 26-29 ‰, pH = 7.8-8.5 and DO = 6.0-8.3 mg/liter. Static and static-renewal (1/3 seawater replaced every third day) systems compared 5, 12 or 20 worms/treatment. Endpoints = mortality, total biomass and average individual biomass. All tests were conducted with aeration and no feeding.

Results:

West Beach and Carr Inlet sediments were essentially non-toxic with mean survivals in all treatments ≥87%. Mean survivals in Elliott Bay sediments ranged from 8 to 44% with highest survivals in the static-renewal system. Mean total biomasses varied with the number of worms added to the chambers and the number surviving to the end of the test. Average individual biomasses were very similar for all treatments, possibly due to the lack of food during the tests. Lack of food may also have acted as a stress in the test.

Neff, J. M., R. S. Carr and W. L. McCulloch. 1980. Acute toxicity of a used chrome lignosulphonate drilling mud to several species of marine invertebrate. *Marine Environ. Res.* 4:251-266.

This study exposed four species of bivalve molluscs and five species of crustaceans to a drilling mud commonly used on Gulf of Mexico drilling platforms. Exposures were to mud aqueous fractions (MAF), filtered MAF (FMAF), layered solid phase (LSP) and suspended solids phase (SSP).

Methods:

The test animals were:

Polychaetes: *Neanthes arenaceodentata*
Ctenodrilus serratus
Ophryotrocha labronica
Dinophilus sp.

Bivalves: *Aequipecten amplicostatus*
Rangia cuneata
Mercenaria mercenaria
Donax variabilis texasiana

Crustaceans: *Penaeus aztecus and P. duorarum*
Palaemonetes pugio
Aysidopsis almyra
Portunus spinicarpus

For LSP, a measured volume of mud was layered over clean natural sediment. SSP bioassays were prepared by adding known volumes of mud to seawater in an aquarium with aeration to keep the particulates suspended. MAF was prepared by mixing mud with seawater at a 1:9 ratio mixing, allowing to settle for 20 hours and siphoning off the supernatant. FMAF was prepared by filtering and centrifuging the MAF. Artificial seawater (Instant Ocean) was used to produce all test solutions and also used as the control seawater. Temperatures in all tests = 22-25 °C, salinities varied from 10 ‰ to 35 ‰, photoperiod = normal day/night cycle and the exposure tanks = various, from 10 cm finger bowls up to 20 liter aquaria. Most tests were static but some were renewal. Exposure times ranged from 1 to 14 days. Test endpoint = survival with subsequent calculations of the LC50s.

Results:

Group	LC50 (% MAF)
Polychaetes	10 to >100
<i>N. arenaceodentata</i>	
Adult static	51
Adult renewal	10
Juvenile MAF	96
Juvenile FMAF	>100

Group	LC50 (% MAF)
Crustaceans	27 to 96
Bivalves	86 to >100

FMAF was slightly less toxic than MAF. Generally, SSP and LSP were less toxic than MAF. Toxicity was greater in the static renewal vs. static exposures. Adult *Neanthes* were generally more sensitive to drilling mud than juveniles. Bivalves were the least sensitive group.

Pesch, C. E. and G. L. Hoffman. 1983. Interlaboratory comparison of a 28-day toxicity test with the polychaete *Neanthes arenaceodentata*. Pp. 482-493 In: Aquatic Toxicology

and Hazard Assessment: Sixth Symposium, W. E. Bishop, R. D. Cardwell and B. B. Heidolph, eds. ASTM STP 802. Am. Soc. for Testing and Materials, Philadelphia, PA.

Six laboratories (2 EPA + 4 contract labs) conducted parallel toxicity tests of silver and endosulfan with *Neanthes* in 28-day exposure tests with endpoints of death (LC50s) and ability to burrow (EC50s).

Test conditions:

1. 28-day exposure in flow-through system with clean sediments
2. Worms fed algae *Enteromorpha*
3. Temp. = 20 ± 1 °C, salinity = 30 ± 2 ‰
4. Silver = AgNO_3 ; Endosulfan = Thiodan, 94.4% pure.

Results:

Based on **measured concentrations**, ratios of highest/lowest LC50 values were 2.23 and 1.81 for silver and endosulfan, respectively. The EC50s (burrowing ability) generally were about the same as the LC50s because most live animals were able to burrow. Paper also gives 96-hr and 10-day LC50s and EC50s. The biggest problem with the interlab comparison was the various lab's abilities to measure the toxicants and dose the tests properly.

Pesch, C.E. and D. Morgan. 1978. Influence of sediment in copper toxicity tests with the polychaete *Neanthes arenaceodentata*. Water Res. 12:747-751.

The authors exposed *Neanthes* to copper in seawater with and without sand in the assay containers for 96 hrs and 28 days. Salinity = 31 ± 1 ‰, temp. = 17 ± 1 °C, in a flow-through bioassay system.

96-hr LC50s = ???? mg/liter copper with and with out sand, respectively. 28-day LC50s = 0.044 & 0.10 mg/liter copper with and without sand, respectively. Tissue copper concentrations were greater in worms exposed without sand in the containers. Concentrations of copper in the sand decreased with depth in the sediment.

Pesch, C.E. 1979. Influence of three sediment types on copper toxicity to the polychaete *Neanthes arenaceodentata*. Marine Biology 52:237-245.

Neanthes exposed to 0.10 mg/liter copper in mud, sand, mud/sand mix and no sediment in a flow-through system for 85 days. Salinity = 32 ± 1 ‰, temp. = 18 ± 1 °C, and worms fed *Enteromorpha* two times/day.

Times to 50% mortality:

Without sediments	7.8 days
Sand	36.5 days
Mud	50.0 days
Sand/mud mix	54.5 days

Sediments probably reduced the amount of bioavailable copper. Copper concentrations in the dead worms exposed without sediments was significantly lower than for those in sediments. Hence, worms in the sediment containers possibly less stressed. Long-term toxicity threshold for copper was <0.10 mg/liter. Data on surfacing of *Neanthes* were also collected.

WATER COLUMN

Cripps, R. A and D. J. Reish. 1973. The effect of environmental stress on the activity of malate dehydrogenase and lactate dehydrogenase in *Neanthes arenaceodentata* (Annelida:Polychaeta). *Comp. Biochem. Physiol. B, Comp. Biochem.* 46(1):123-133.

The purpose of this study was to determine whether or not environmental stress can cause changes at the enzymatic level in the polychaete *N. arenaceodentata*. Specifically, the authors investigated the effects of lowered dissolved oxygen and hyper- and hypo-saline conditions on MDH and LDH activities.

Methods:

Individual Reish-cultured worms were exposed for 10 days in 100 ml natural seawater contained in 250 ml flasks at 18 ± 1 °C with *Enteromorpha* supplied as food. DO levels were controlled by flushing the flasks with appropriate amounts of nitrogen gas.

Results:

Lowered DOs caused initial increases in MDH (both cytosol and mitochondrial) and LDH activities; further reductions in oxygen levels resulted in decreases in activities of LDH and cytosol MDH. Mitochondrial MDH continued to increase in activity down to the lowest DO levels. The ratio of total MDH to LDH activities increased about 56% with lowered DOs.

Low salinity levels of 25 % had little effect on MDH (both cytosol and mitochondrial) activities. Hypersalinity caused an increase in mitochondrial MDH activity, but had no significant effect on cytosol MDH. Salinity fluctuations had a marked effect on LDH activities.

Davis, W. R. and D. J. Reish. 1975. The effect of reduced dissolved oxygen concentration on the growth and production of oocytes in the polychaetous annelid, *Neanthes arenaceodentata*. *Rev. Intern. Océanogr. Méd.* 37/38:3-16.

The authors exposed *N. arenaceodentata* to reduced dissolved oxygen concentrations to investigate its effects on worm survival and oocyte development, number and growth.

Methods:

Individual young female worms were exposed to reduced levels of dissolved oxygen in 500 ml flasks by flushing the sealed flasks with varying amounts of nitrogen gas. Exposure time = 56 days, worms were fed *Enteromorpha* and the temperature = 14-16 °C.

Results:

Endpoint	Dissolved Oxygen (mg/liter)				
	5.9	3.0	2.0	1.0	0.5
% Survival	100	100	90	60	40
Oocyte growth (μm)	433	325	220	75	70
Oocyte number	139	139	75	15	5

Thus, survival and oocyte number decreased at ≤ 2.0 mg/liter DO and oocyte growth decreased at ≤ 3.0 mg/liter DO. The 56 day LC50 was 0.6 mg/liter DO.

Jenkins, J. D. and B. M. Sanders. 1986. Relationships between free cadmium ion activity in seawater, cadmium accumulation and subcellular distribution, and growth in polychaetes. *Environ. Health Perspectives* 65:205-210.

Neanthes arenaceodentata were exposed for 3 wks to ionic cadmium (Cd^{2+}) in 35‰ seawater at 6 different Cd^{2+} concentrations. Buffers were used to maintain the Cd^{2+} concentrations at the appropriate levels. ^{109}Cd was used as a tracer. Metal/seawater concentrations were renewed at 1-wk intervals. Temperatures and pHs were not given. Sub-cellular components were analyzed for Cd concentrations at the end of the test and growth monitored at weekly intervals.

Results:

The data revealed a linear relationship between Cd accumulation and the Cd^{2+} concentrations in exposure seawater, indicating that Cd accumulation was a simple function of bioavailability (no short-term regulation of Cd uptake by the worms). Most of the Cd was associated with the cytosol. It is possible that there was some long-term regulation of Cd associated with metallothioneine-like components. Growth increased in moderate concentrations of Cd and decreased at the highest concentration, thus showing a hormetic response to Cd.

Mason, A. Z., K. D. Jenkins and P. A. Sullivan. 1988. Mechanisms of trace metal accumulation in the polychaete, *Neanthes arenaceodentata*. *J. Marine Biol. Assn. U. K.* 68:61-80.

The authors exposed juvenile *Neanthes* to radioactive labeled zinc and cadmium to study the passive uptake, elimination and partitioning of these metals at high (21 °C) and low (4 °C) temperatures with and without the presence of metabolic inhibitors.

Methods:

Worms were exposed to ^{65}Zn and ^{109}Cd in the presence of the metal chelator EDTA. *Neanthes* were exposed for 36 or 360 hours in 30 ml of filtered seawater (salinity = 35 ‰) in petri dishes at 21 or 4 °C, without feeding during the exposures.

Results:

Zn and Cd uptake during the first 36 hrs were essentially linear for all groups. Metabolically inhibited worms showed greater accumulations, suggesting that the elimination of Zn and Cd was energy-dependent. Zn was primarily accumulated in the soft parts and Cd in the jaws.

Mearns, A. J., P. S. Oshida, M. J. Sherwood, D. R. Young and D. J. Reish.
1976. Chromium effects on coastal organisms. *J. Water Poll. Cont. Fed.* 48(8):1929-1939.

This work exposed the polychaete, *Neanthes arenaceodentata* and speckled sanddabs, *Citharichthys stigmaeus* to trivalent and hexavalent chromium in natural seawater. *Neanthes* testing included long-term, multi-generation (up to 160 days) exposures.

Methods:

Sanddabs were trawled from Santa Monica Bay and exposed to Cr in aerated 20 gal aquaria with static and continuous-flow systems, temp. = 12-13 °C and exposure times = 4 & 21 days. *Neanthes* were exposed in short-term (7 days) static tests using 1 worm (30-40 segments)/100 ml of solution in 500 ml flasks, food = *Enteromorpha*. Long-term tests ran as long as 350 days in 1-gal glass jars with single or pairs of worms. Test solutions were replaced at 2-3-week intervals, temp. = 20 ± 0.6 °C, salinity = 33.5 ‰, pH = 7.8-8.0, DO $\geq 75\%$ saturation, with 20 replicates/concentration.

Results:

For sanddabs, 4 and 21-day LC50s were 30 and 5 mg/liter for chrome VI, respectively. Feeding responses were affected at $\sim 1/2$ of the LC50s. Chrome III produced no mortalities with most of the chrome III present as precipitates.

For *Neanthes*, chrome VI LC50s were 3.1, 1.63 and ~ 0.20 mg/liter for 4, 7 and 59 days, respectively. There was an inhibition of mucus production at 0.23 mg/liter for the 7-day test. For long-term tests, eggs failed to be laid at 0.2 and 0.1 mg/liter chrome VI and there was a decrease of brood size at 0.0125 mg/liter. For chrome III, no toxicity was observed in 160-day exposures, even though the worms were ingesting the Cr III precipitates.

Oshida, P. S., L. S. Word and A. J. Mearns. 1981. Effects of hexavalent and trivalent chromium on the reproduction of *Neanthes arenaceodentata* (Polychaeta). *Marine Environ. Res.* 5:41-49.

Methods:

Neanthes were exposed to two forms of chromium in acute 7-day tests and in sublethal 3-generation tests in a static-renewal (3-week intervals) system. Mean salinity = 33.6 ‰, temp. = 20 ± 0.6 °C, DO = 7.1 mg/liter, pH = 7.9, and worms were fed *Enteromorpha* weekly in long-term tests.

Results:

The 7-day LC50 for both wild stock and laboratory worms = 1.46 to 1.78 mg/liter hexavalent chromium. No significant difference in sensitivity was detected between wild and laboratory worms. For the long term tests, there was a significant difference in brood sizes at 12.5 $\mu\text{g/liter}$ Cr VI in the parental generation, in 25 $\mu\text{g/liter}$ in the F1 generation and in 50 $\mu\text{g/liter}$ in the F2 generation. Thus, there was less sensitivity to Cr VI with longer exposures. There was no toxicity of Cr III up to 50.4 mg/liter (>99% occurred as precipitate in the bioassay containers and was eaten by the worms without any negative effects).

Oshida, P. S. and L. S. Word. 1982. Bioaccumulation of chromium and its effects on reproduction in *Neanthes arenaceodentata* (Polychaeta). *Marine Environ. Res.* 7:167-174.

Neanthes were exposed to hexavalent chromium for 2 generations (309 days) at concentrations of 1 to 38 $\mu\text{g/liter}$. Salinity = 33-35 ‰, temp. = 18-24 °C, pH = 8.09, and worms were fed *Enteromorpha*. A static renewal system (toxicant renewed at 3-wk intervals) was used. Test endpoints were time to spawning, brood size of the P and F1 generations and whole-body bioaccumulation of chromium.

Results:

- 1) Worms fed and behaved normally at all test concentrations
- 2) No effects on time-to-spawning
- 3) Brood size of P generation increased at 16.6 $\mu\text{g/liter}$ Cr VI
- 4) Brood size of F1 generation decreased at 38.2 $\mu\text{g/liter}$ Cr VI
- 5) Worms accumulated Cr VI up to 8,278 $\mu\text{g/wet Kg}$ in P generation and up to 6,030 $\mu\text{g/wet Kg}$ in the F1 generation
- 6) Bioaccumulation of up to 8,000 $\mu\text{g/wet Kg}$ had no effect on reproductive success of the P generation.

Oshida, P. S. 1976. Effects of chromium on reproduction in polychaetes. Pp. 161-167 *In:* Coast. Water Res. Proj. Annual Rpt. 1976. South. Calif. Coast. Water Res. Proj., El Segundo, CA. 263 pp.

Neanthes were exposed to chromium III and VI for 293 and 440 days, respectively.

For chromium VI:

LT50s: 59 days in 0.2 mg/liter
 184 days in 0.1 mg/liter
 >440 days at concentrations \leq 0.05 mg/liter

Brood sizes were significantly less at 0.0125 mg/liter in P1 generation.

Brood sizes were significantly less at 0.05 mg/liter in F1 & F2 generations.

The 7-day LC50s for field-collected and laboratory-cultured worms (P, F1 & F2 generations) were all in range of 1.44 to 1.89 mg/liter with no obvious differences in sensitivities. Accumulation of chromium VI was proportional to the exposure concentrations.

For chromium III:

Chromium III essentially non-toxic at 0.2 mg/liter.

Oshida, P. S. 1977. A safe level of hexavalent chromium for a marine polychaete. Pp. 169-180 *In: Coast. Water Res. Proj. Annual Rpt. 1977.* South. Calif. Coast. Water Res. Proj., El Segundo, CA. 253 pp.

This paper reports the same results of 2-generation (309-day) worm exposures to chromium VI as reported in Oshida and Word (1982; *Marine Environ. Res.* 7:167-174).

Oshida, P. S. and D. J. Reish. 1974. The effect of various water temperatures on the survival and reproduction in polychaetous annelids: Preliminary report. Pp. 63-76 *In: Marine Studies of San Pedro Bay, California. Part III. Thermal Tolerance and Sediment Toxicity Studies*, D.F. Soule and M. Oguri, eds. Alan Hancock Foundation, Harbors Environmental Projects. USC-SG-1-74.

Neanthes were exposed to temperatures of 5, 9, 13, 17, 23, 26, 29, 32 and 35 °C for 28 days and in long-term (up to F2 generation) tests.

Lower TLm (28-day) = 12.3 °C
Upper TLm (28-day) = 24.6 °C

High mortalities were observed in 5, 9, & 35 °C

"To date" (experiment still in progress), eggs were only laid in the 23 °C group. Worms in the 13 & 17 °C groups lived but did not produce eggs (yet).

Pesch, G. G. and C. E. Pesch. 1980. *Neanthes arenaceodentata* (Polychaeta:Annelida), a proposed cytogenetic model for marine genetic toxicology. *Can. J. Fish. Aquatic Sci.* 37:1225-1228.

This paper describes an *in vivo* sister chromatid exchange (SCE) assay with *Neanthes*.

The authors found a dose-response effect following exposures to mitomycin C (MMC). Rates of SCE were similar to mouse and rabbit systems. More development of the test is required for a refined technique.

Pesch, C. E. and G. L. Hoffman. 1982. Adaptation of the polychaete *Neanthes arenaceodentata* to copper. *Marine Environ. Res.* 6:307-317.

Neanthes were exposed to sub-lethal concentrations of copper (10-28 µg/liter) for 27 days and then subjected to an LC50 assay at 56-292 µg/liter copper for 28 days. Testing used a flow-through system with a salinity of $31 \pm 1 \%$, temperature = 19 ± 1 °C, and worms fed *Enteromorpha* 2X/week.

Worms pre-exposed to the highest copper concentration (28 $\mu\text{g/liter}$) were significantly more tolerant to copper than worms exposed to the other lower concentrations. The pre-exposure concentration also seemed to affect tissue copper accumulations. The possible interaction with metallothionein is unknown.

Pesch, C. E., R. N. Zajac, R. B. Whitlatch and M. A. Balboni. 1987. Effect of intraspecific density on life history traits and population growth rate of *Neanthes arenaceodentata* (Polychaeta:Nereidae) in the laboratory.

This study raised *Neanthes* in laboratory test chambers at three different densities to assess the effects on survival, growth and reproduction. A concurrent goal was to develop a laboratory experimental system, with *Neanthes* as the test species, to assess population level responses to toxicants and contaminated sediments.

Methods:

Neanthes were raised at three different densities (40, 80 and 160 worms/840 cm^2) in 33.6 X 25 X 15.5 cm Plexiglas boxes provided with flow-through, sand-filtered seawater (70 ml/min), temperature = $19 \pm 1^\circ\text{C}$, salinity = 30-32 ‰ and photoperiod = 10 hr:14 hr light:dark. Worms were provided powdered prawn flakes for food (on a constant food weight/worm basis) and ground, dried *Enteromorpha* for tube building. The experimental design used 3 replicates/treatment and included a "handling" control. The test was terminated at day 70.

Results:

Worm density did not affect growth (prior to pairing), percentage of worms paired, time to pairing, or the size of mature paired males. Density did have a significant negative effect on survival, size of mature paired females, time to spawning, percentage of females that reproduced and number of eggs per reproducing female. As density increased, mean survival was 90.0, 80.8 and 74.0%; mean size of mature females was 52.2, 49.2 and 48.1 segments; mean time to spawning was 100.6, 102.4 and 109.4 days; and mean fecundity was 881, 622 and 598 eggs/female for 40, 80 and 160 worms/840 cm^2 , respectively.

The results of this test provided valuable background data for eventual use of life-cycle testing with pollutants.

Petrich, S. M. and D. J. Reish. 1979. Effects of aluminum and nickel on survival and reproduction in polychaetous annelids. Bull. Environ. Contam. Toxicol. 23:698-702.

Three species of worms were exposed to chloride salts of aluminum (AlCl_3) and nickel (NiCl_2) in 96-hr and 7-day tests. *Ctenodrilus serratus* was also exposed for a complete life cycle (28 days).

Results (LC50s, mg/liter):

Species	AlCl ₃		NiCl ₂	
	96-hr	7-day	96-hr	7-day
<i>Neanthes arenaceodentata</i>	>2.0	>2.0	49	17
<i>Capitella capitata</i>	2.0	>2.0	>50	>50
<i>Ctenodrilus serratus</i>	0.48	----	17	----

Chronic life-cycle results with *Ctenodrilus* showed that reproductive suppression and LC50 values were at essentially identical concentrations for aluminum. Reproductive effects in nickel were at concentrations 1-2 orders of magnitude less than the 96-hr LC50.

Raps, M. E. and D. J. Reish. 1971. The effects of varying dissolved oxygen concentrations on the hemoglobin levels of the polychaetous annelid *Neanthes arenaceodentata*. Mar. Biol. 11(4):363-368.

The authors exposed *Neanthes* to reduced levels of dissolved oxygen for periods up to 19 days to determine if hemoglobin compensation at low DO levels takes place in annelids.

Methods:

Neanthes were individually exposed to reduced DO concentrations in 100 ml of seawater in stoppered flasks. DO concentrations were controlled by flushing the flasks with nitrogen gas. *Enteromorpha* was supplied as a food source. Pre-exposure culture conditions were: temperature = 24 ± 1 °C, salinity = 35 ± 1 ‰ and DO = 7.3 ± 0.3 mg/liter. Hemoglobin concentrations were measured colorometrically.

Results:

Hemoglobin compensation was found to take place in *Neanthes*; this was the first indication of compensation in this phylum. Compensation was initiated at about 4.2 mg/liter DO, significantly increased at 3.0 mg/liter and continued to increase down to lethal DO levels. Cages of worms were also set out in four places in Los Angeles Harbor. Hemoglobin concentrations increased slightly in these animals and were associated with reduced DO levels.

Reish, D. J. 1970. The effects of varying concentrations of nutrients, chlorinity, and dissolved oxygen on polychaetous annelids. Water Res. 4:721-735.

Four species of polychaetes were exposed to increased nutrients, decreased salinity (chlorinity) and decreased dissolved oxygen (DO) for 28 days and 28-day median Lethal Times (TL_m) were calculated.

Results:

Factor	<i>Nereis grubei</i>	<i>Neanthes arenaceodentata</i>	<i>Dorvillea articulata</i>	<i>Capitella capitata</i>
Phosphate (μg-at/liter)	920	1900	2100	2400
Nitrate "	5300	8000	14,200	11,500
Silicate "	250	>250	>250	210
Chlorinity (%)	13.5	11.2	12.6	10.5
DO (mg/liter)	2.95	0.90	0.65	1.50

Reish, D. J. 1974. The sublethal effects of environmental variables on polychaetous annelids. Rev. Intern. Océanogr. Méd. 33:83-90.

The author exposed the polychaete *Neanthes arenaceodentata* to varying concentrations of dissolved oxygen (controlled with N₂ flushing) in seawater. The mortality endpoint was compared to a variety of sublethal endpoints. *Neanthes* were exposed for 7-56 days in sealed flasks in 500 ml seawater flushed with N₂ to maintain desired O₂ levels. Worms were fed *Enteromorpha*.

Results:

- 28 day TL₅₀ = 0.90 mg/liter DO
- 50% reduced feeding = 0.95 mg/liter
- 50% reduced egg production = 2.0 mg/liter
- Change in MDH/LDH enzyme levels = ~2.5 mg/liter
- Significant change in free amino acid concentrations = 2.5 mg/liter
- Increased hemoglobin compensation = 4.5 mg/liter.

Reish, D. J. 1978. The effects of heavy metals on polychaetous annelids. Rev. Intern. Océanogr. Méd. 46:99-103.

This is a summary review paper on the effects (acute and reproductive) of six metals to four species of polychaetes.

Some results:

Species	Metal — mg/liter					
	Cd	Cr	Cu	Pb	Hg	Zn
<i>Neanthes arenaceodentata</i>						
96-hr LC50	12.1	3.2	0.3	7.7	0.02	1.8
Reprod. failure	3.2	0.1	----	3.1	-----	1.8

Species	Metal — mg/liter					
	Cd	Cr	Cu	Pb	Hg	Zn
<i>Capitella capitata</i>						
96-hr LC50	5.8	5.0	0.2	11.4	<0.1	10.7
Reprod. failure	>1.0	>0.4	----	1.3	-----	3.2
<i>Ctenodrilus serratus</i>						
96-hr LC50	>20.0	4.3	0.3	14.0	0.09	7.1
Reprod. failure	5.0	1.0	0.25	5.0	0.1	10.0
<i>Ophryotrocha diadema</i>						
96-hr LC50	4.2	>5.0	0.16	14.0	0.09	2.7
Reprod. failure	2.5	1.0	0.25	0.5	0.1	1.75

Neanthes was the most sensitive species to metals and *Ctenodrilus* most tolerant.

Reish, D. J. 1980. The effect of different pollutants on ecologically important polychaete worms. EPA Tech. Rpt. EPA-600/3-80-053. 138 pp.

This paper gives culture techniques for 12 species of polychaetes:

- 1) *Neanthes arenaceodentata*
- 2) *Capitella capitata*
- 3) *Ctenodrilus serratus*
- 4) *Ophryotrocha diadema*
- 5) *O. puerilis*
- 6) *Dexiospira brasiliensis*
- 7) *Polydora ligni*
- 8) *Boccardia proboscidea*
- 9) *Dinophilus* sp.
- 10) *Cirriformia luxuriosa*
- 11) *C. spirabrancha*
- 12) *Halosydna johnsoni*

Effects of six heavy metals on polychaete survival:

For *Neanthes*:

Metal (mg/liter)	96-hr LC50		28-day LC50	
	Adult	Juvenile	Adult	Juvenile
Cadmium	12.0	12.5	4.0	3.0
Chromium	1.0	1.0	0.55	0.7
Copper	0.3	0.3	0.25	0.14
Lead	10.0	7.5	3.2	2.5
Mercury	0.22	0.1	0.17	0.09
Zinc	1.8	0.9	1.4	0.9

Also, chromium had a significant effect on reproduction at 0.0125 mg/liter.

Reish, D. J., J. M. Martin, F. M. Piltz and J. Q. Word. 1976. The effect of heavy metals on laboratory populations of two polychaetes with comparisons to the water quality conditions and standards in Southern California marine waters. *Water Res.* 10:299-302.

The authors exposed *Neanthes* and *Capitella capitata* to six metals in seawater for 28 days to determine 96-hr and 28-day LC50s.

Results:

Metal (mg/liter)	<i>Neanthes</i>				<i>Capitella</i>		
	96-hr LC50 Adult	96-hr LC50 Juvenile	28-day LC50 Adult	28-day LC50 Juvenile	96-hr LC50 Adult	96-hr LC50 Trophophore	28-day LC50 Adult
Cadmium	12.0	12.5	3.0	3.0	7.5	0.22	0.7
Chromium	>1.0	>1.0	0.55	0.7	5.0	8.0	0.28
Copper	0.3	0.3	0.25	0.14	0.2	0.18	0.2
Lead	>10.0	>7.5	3.2	2.5	6.8	1.2	1.0
Mercury	0.022	0.1	0.017	0.09	<0.1	0.014	0.1
Zinc	1.8	0.9	1.4	0.9	3.5	1.7	1.25

Rossi, S. S. and J. W. Anderson. 1976b. Toxicity of water-soluble fractions of No. 2 fuel oil and south Louisiana crude oil to selected stages in the life history of the polychaete, *Neanthes arenaceodentata*. *Bull. Environ. Contam. Toxicol.* 16(1):18-24.

The authors exposed juvenile, adult, male and female *Neanthes* to various concentrations of water-soluble fractions (WSF) of two types of oil.

Methods:

Juvenile (4, 18, 32 & 40 segments) and adult (60 segments) worms were exposed to 0-100% concentrations of WSF. For juveniles, 10 worms were exposed in 100-ml culture dishes containing 50 ml of WSF. Adults were assayed in 125 ml flasks with 50 ml WSF. Each assay was repeated 4 times. Temperature = 22 ± 1 °C, salinity = 32‰ (Instant Ocean) and the exposure time = 96 hrs.

Results:

No mortalities were observed in the controls. No. 2 fuel oil was more toxic to both the juveniles and the adults than the crude oil. The toxicity of WSF increased with the size of the worms. The possible explanation for this was that the higher yolk content of the juveniles provided some protection from toxicity. Males and females were equally sensitive.

Rossi, S. S., J. W. Anderson and G. S. Ward. 1976b. Toxicity of water-soluble fractions of four test oils for the polychaetous annelids, *Neanthes arenaceodentata* and *Capitella capitata*. Environ. Poll. 10:9-17.

Neanthes and *Capitella* exposed to four types of oil (water-soluble fractions—WSF). Nine parts Instant Ocean to 1 part oil (stirred slowly for 20 hours) = 100% WSF. Test temp. = 20 ± 1 °C, salinity = 32 %. Test endpoints were 24, 48 and 96-hr LC50s.

Results (tabular values in mg/liter Total Hydrocarbons):

Oil Type (WSF)	<i>Neanthes</i>			<i>Capitella</i>		
	24 hr	48 hr	96 hr	24 hr	48 hr	96 hr
No. 2 fuel oil	>8.7	3.2	2.7	>8.7	3.5	2.3
Bunker "C"	>6.3	4.6	3.6	>6.3	1.1	0.9
South LA crude	18.0	13.9	12.5	>19.8	16.2	12.0
Kuwait crude	>10.4	>10.4	>10.4	>10.4	>10.4	9.8

Note that *Capitella* was more sensitive than *Neanthes*. Primary toxicants in the oils probably naphthalenes, benzenes and possibly phenols.

Rossi, S. S. and J. W. Anderson. 1977a. Accumulation and release of fuel-oil-derived diaromatic hydrocarbons by the polychaete *Neanthes arenaceodentata*. Marine Biology 39:51-55.

This study exposed *Neanthes* to sublethal concentrations of water soluble fraction (WSF) of No. 2 fuel oil for 24 hrs and monitored hydrocarbon (esp. naphthalenes) uptake and subsequent depuration during post-exposure.

Methods:

Reish-cultured male and gravid female *Neanthes* (50-75 mg wet wt) were exposed collectively to 25% WSF of No. 2 fuel oil for 24 hrs in 3 liter glass aquaria. Depuration was carried out in 300 ml culture dishes for up to 500 hrs post-exposure. Dilution water = Instant Ocean at 22 ± 1 °C and 32 % salinity. Hydrocarbon concentrations were measured by UV spectrophotometry.

Results:

Both male and female worms accumulated hydrocarbons very rapidly, with maximum uptake occurring within 1 hr of initial exposure. No depuration occurred during the 24 hr exposure period, but depuration was rapid in the males post-exposure, with the majority of the naphthalenes depurated within 72 hrs and to <0.1 mg/liter in 400 hrs.

Gravid females retained most or all of the naphthalenes until spawning, indicating that the hydrocarbons were rapidly sequestered in the eggs. Zygotes and trochophores of exposed worms

contained up to 18 mg/liter of total naphthalenes. Subsequent growth by the worms was associated with fairly rapid depuration.

Rossi, S. S. and J. W. Anderson. 1977b. Effect of No. 2 fuel oil and South Louisiana crude oil water-soluble fractions on hemoglobin compensation and hypoxia tolerance in the polychaetous annelid, *Neanthes arenaceodentata* (Moore).

Neanthes were exposed to water-soluble fractions (WSFs) of fuel and crude oils at low dissolved oxygen concentrations to investigate effects on worm survival and the "hemoglobin compensation" response.

Methods:

Neanthes were immature male and females from the Reish culture system. Individual worms were exposed to 75 ml WSF in 250 ml flasks for 11 days with *Enteromorpha* added as food. Twenty worms were used/variable. DO concentrations were controlled by nitrogen flushing. Dilution water = Instant Ocean at 22 ± 1 °C and 32 % salinity. Oil concentrations were measured by UV and IR spectrophotometry.

Results:

Neanthes could survive in DO concentrations as low as 2.0 mg/liter (without oil). The approximate 96 hr LC50 concentration of No. 2 fuel oil was 32% WSF at high (~7 mg/liter) DO levels. The 96 hour LC50 for South Louisiana crude oil was about 63% WSF (from previous work). Low DO concentrations markedly increased toxicity of South Louisiana crude oil WSF's, producing a synergistic effect. Low DOs did not alter the toxicity of No. 2 fuel oil. However, neither oil WSF caused a disruption of the hemoglobin compensation response.

Rossi, S. S. and J. W. Anderson. 1978. Effects of No. 2 fuel oil water-soluble-fractions on growth and reproduction in *Neanthes arenaceodentata* (Polychaeta:Annelida). Water, Air, and Soil Poll. 9:155-170.

The authors exposed *Neanthes* to various concentrations of oil WSF at various stages in its life cycle and assessed multiple endpoints.

Reproduction (brood mortality and zygote production) was the most sensitive indicator of toxicity followed by juvenile growth and then larval growth. Reproductive success and juvenile growth were both affected in a dose-responsive fashion to all WSF concentrations tested (lowest WSF concentration = 2.5%). An important conclusion was that several life history stages must be investigated to define chronic toxicity to marine organisms.

Rossi, S. S. and J. M. Neff. 1978. Toxicity of polynuclear aromatic hydrocarbons to the polychaete *Neanthes arenaceodentata*. Marine Poll. Bull. 9:220-223.

This study exposed *Neanthes* to varying concentrations of specific polynuclear aromatic hydrocarbons (PNA's) to determine toxicities in terms of 96-hr LC50s.

Methods:

Immature young adult *Neanthes* from the Reish culture system were exposed for 96 hrs to 50 ml of unaerated PNA/seawater solutions in 125 ml flasks. PNA carrier = acetone. Tests used 10 animals/concentration with 2 tests/PNA species, dilution water = Instant Ocean at 22 ± 2 °C and 32‰ salinity. PNA concentrations were measured by UV spectrophotometry. Worms were not fed during the exposure periods.

Results:

Solubilities of PNA's in seawater varied by species with solubilities ranging from 0.005 to 20 µg/g. Solubilities and toxicities were as follows:

PNA	Solubilities (µg/g)	Toxicity 96-hr LC50 (mg/liter) (as initial PNA conc.)
Naphthalene	20.0	3.8
Dimethylnaphthalene	2.4	2.6
Trimethylnaphthalene	1.7	2.0
Fluorene	0.8	1.0
Phenanthrene	0.6	0.6
Methylphenanthrene	0.3	0.3
Fluoranthene	0.01	0.5
Chrysene	0.001-0.05	>1
Benzo (a) pyrene	0.005-0.01	>1
Dibenzanthracene	0.005-0.01	>1

Solubilities of PNA's were closely related to molecular weight (MW) and varying inversely with molecular weight. PNA toxicity was also related to MW with a trend towards increased toxicity with increased MW. There were significant decreases in PNA concentrations in the flasks over the first 48 hours, probably due to volatilization and/or photo-oxidation.

TAXONOMY, CULTURE, AND MISCELLANEOUS INFORMATION

Pesch, G. G. and C. E. Pesch. 1980. Chromosome complement of the marine worm *Neanthes arenaceodentata* (Polychaeta:Annelida). *Can. J. Fish. Aquatic Sci.* 37:286-288.

The chromosome complement of *Neanthes* consists of 18 diploid chromosomes (9 pairs) ranging in size from 4-7 µm. A method is described for fixing, processing and staining material for chromosome investigations. *Neanthes* is being examined for use in a Sister Chromosome Exchange (SCE) genotoxicity assay.

Pesch, G. G., C. E. Pesch and C. Mueller. 1988. Chromosome complements from two populations of the marine worm *Neanthes arenaceodentata* (Annelida:Polychaeta). *Ophelia* 28(2):163-167.

The authors investigated the chromosome complements and morphologies of *Neanthes* from Southern California and Connecticut populations.

Methods:

Worms from Don Reish's lab in Southern California (CSU, Long Beach—worms were originally from Los Angeles Harbor) were compared with worms from the lab of Roman Zajac (worms originally from Alewife Cove, Connecticut). Chromosomes were prepared by a standard preparation and staining process.

Results:

The California worms had a diploid chromosome number of 18 vs. 24 for the Connecticut worms and there were also differences in the chromosome morphologies. Thus, these animal's are clearly different species. The authors suggest that the East Coast species retain the name *Neanthes arenaceodentata* and that renaming of the West Coast species be considered.

Reish, D. J. 1957. The life history of the polychaetous annelid *Neanthes caudata* (delle Chiaje), including a summary of development in the Family Nereidae. *Pacific Science* 11(2):216-228.

Neanthes caudata = *Neanthes arenaceodentata*

This species was first found in the Pacific by Don Reish in Long Beach Harbor in 1953. It is thought to be a native of Europe and possibly an exotic species in California.

Some characteristics:

- Worms cannibalistic and sexes fight same sex
- Males incubate eggs in mucoid tubes, female dies or is eaten after egg laying
- Sex ratio 1:1
- Will eat dried *Enteromorpha* and other dried food sources
- Number of eggs per brood = 143-791
- Eggs have fertilization membranes
- Juveniles leave brood tube at about 16 days and 16 segments
- This article gives a detailed schedule of development

Reish, D. J. 1973. Laboratory populations for long-term toxicity tests. *Marine Poll. Bull.* 4(1):46.

Lab-cultured animals assist the goal of international standardization of bioassay tests.

Laboratory cultures:

- Reduce stress
- Available when needed
- Animals adapted to lab conditions
- Known diet

- No decimation of field populations
- Worms have short life-histories and easy to transport

Reish, D. J. 1974. The establishment of laboratory colonies of polychaetous annelids. *Thalassia Jugoslavica* 10 (1/2):181-195.

This article generally discusses the use of polychaetes for basic biological studies and the potential for establishing laboratory cultures. It provides a summary table on life history studies of polychaetes and discusses the following topics:

- Important aspects of cultured species
- Reproduction and spawning
- Zygotes and larvae
- Food and feeding

This paper also gives specifics for culturing and handling *Capitella capitata*.

Reish, D. J. 1989. Bibliography of *Neanthes arenaceodentata*. Unpublished list of references by D. J. Reish, Department of Biology, Calif. State Univ., Long Beach, CA. 90840. 9 pp.

This is the most complete bibliographic listing available for work conducted on *N. arenaceodentata*. This list is updated periodically by Reish. Several paragraphs regarding taxonomic status of this species are included at the end of the listings.

RELATED POLYCHAETE SPECIES TESTING

Åkesson, B. 1980. The use of certain polychaetes in bioassay studies. *Rapp. P.-v. Réun. Cons. int. Explor. Mer.* 179:315-321.

This article reviews information on use of three worm species for bioassay:

Ophryotroca labronica
Dinophilus gyrociliatus
Dorvillea sp.

This paper summarizes the toxicity of phenol to five worm species (mg/liter):

<i>O. labronica</i>	350 \pm 5
<i>O. notoglandulata</i>	340 \pm 5
<i>O. macrovifera</i>	240 \pm 10
<i>O. robusta</i>	110 \pm 10
<i>O. diadema</i>	110 \pm 5

The article provides a detailed review for *Dorvillea* including starvation, reproduction, age, and temperature/salinity requirements.

Reish, D. J., F. Piltz, J. M. Martin and J. Q. Word. 1974. Introduction of abnormal polychaete larvae by heavy metals. *Marine Poll. Bull.* 5(1):125-126.

The authors exposed developing worms (*Capitella capitata*) to copper and zinc for 1 or 2 generations and noted the incidence of abnormal larvae production.

Methods:

Test toxicants = CuSO₄ and ZnSO₄. Larvae through adult worms were exposed to copper concentrations of 0.01 to 0.05 mg/liter and to zinc concentrations of 0.05 and 0.1 mg/liter in gallon jars, 50-75 larvae or worms per concentration. Larvae and worms were fed *Enteromorpha*.

Results:

Larval abnormalities (primarily bifurcated larvae) were induced by copper following F₁ exposure and by zinc following F₁ + F₂ exposures. Copper produced percent abnormalities of up to 0.9% (F₁) and zinc produced up to 0.35% abnormalities following F₂ exposure. Abnormal larvae always failed to settle and survive.

CHAPTER 5. MICROTOX

METHODOLOGY

Beckman Instruments. 1979. A fast, quantitative toxicity monitor. Chemical Engineering, July 30, 1979:39-40.

This is a short write-up under "New Products & Services" section which describes the new Microtox Monitoring System that is represented as a quick (~10 min) and sensitive alternative to standard 96-hr fish bioassays. This test system exposes luminescent bacteria to toxicants and measures the resulting light outputs. Toxicants usually cause decreased light emissions. The bacteria are lyophilized (= Microtox "reagent") for off-the-shelf convenience

Microbics Corporation. Undated. The Microtox answer man. Answers to common questions about Microtox. Unpublished questions and answers about the Microtox System by Microbics Corp., 222 Rutherford Road, Carlsbad, CA 92008. 2 pp. Reprinted from Microtox World.

Questions and answers cover areas of light stimulation, use with freshwater and marine samples, effects of nutrients, viability and culture of the bacteria "reagent", effect of temperature, EPA approval, etc.

SEDIMENTS

Schiewe, M. H., E. G. Hawk, D. I. Actor and M. M. Krahm. 1985. Use of a bacterial assay to assess toxicity of contaminated marine sediments. *Can. J. Fish. Aq. Sci.* 42(7):1244-1248.

The authors used the Microtox bacterial bioluminescence assay to assess the relative toxicity of 18 natural sediments collected from Puget Sound. This study used an organic solvent extraction procedure instead of a seawater extraction. To support the use of solvent extraction, various candidate solvents were also assessed for toxicity.

Methods:

Sediments were collected from 18 Puget Sound sites by 0.1 m² van Veen grab and the top 2 cm only collected. Samples were frozen at -20 °C until used. Extracts were prepared by washing 100 g of sediment with dichloromethane and methanol by tumbling a total of 24 hrs. Extracts were measured for selected organic compounds and metals.

Bioassays exposed *Photobacterium phosphoreum* to sediment extracts for 5 to 30 min at 15 °C in a 2% NaCl matrix. The test endpoint = 15 min light reduction relative to controls and the subsequent calculation of EC50s. Various solvents were also tested for toxicity.

Results:

Solvent toxicities (15 min EC50s in $\mu\text{g}/\text{ml}$) were:

Dichloromethane	2.15
Acetone	18.35
Methanol	18.62
Ethanol	29.11
DMSO	58.39

Based on these data, ethanol was selected as the solvent of choice; ethanol was "solvent-exchanged" with dichloromethane prior to testing. DMSO was not selected because it's toxicity was markedly increased following solvent exchange, cause unknown.

Toxicities of the 18 Puget Sound sediments ranged from 0.14 to 14.48 $\mu\text{l}/\text{ml}$. The most toxic sediments were from the Hylebos and Duwamish Waterways and Eagle Harbor. Least toxic were sediments from Clinton, Carkeek Park and Richmond Beach. There were statistically significant correlations between the EC50s and concentrations of aromatic hydrocarbons ($r = 0.828$), total naphthalenes ($r = 0.796$) and chlorinated hydrocarbons ($r = 0.669$).

Varanasi, U., S. Chan, D. Brown, R. Clark and E. Casillas. 1989. Summary report for the Olympia Harbor Navigation Improvement Project, 1988: Sediment characterization and Microtox assays. Summary Report by NMFS/NOAA, Northwest Fisheries Center, Seattle, WA. 7 pp. + Appendix Tables and Figures.

This project determined the chemical concentrations and toxicity (via Microtox) of 15 sediments collected from a proposed dredging area near Olympia, WA.

Methods:

Sediment samples were collected by vibracorer from 15 stations at the Port of Olympia and stored at 4-6 °C until analyzed (within 2 weeks). Samples were measured for priority organic and metal contaminants. Subsamples were tested by the Microtox assay system via the PSEP protocol: 15 min EC50s were calculated for saline extracts in a 2% NaCl matrix at 15 °C. The control sediments were from West Beach, Whidbey Island, WA.

Results:

Almost all organic chemicals were below PSDDA screening level (SL) concentrations. For metals, none exceeded PSDDA maximum levels (ML). SLs were exceeded in some samples by nickel, cadmium, copper and mercury.

Microtox testing showed that 3/15 sediments gave a significant toxic response with the EC50s ranging from 0.7 to 2.1 g sediment equivalents (on a dry weight basis)/ml of test solution.

WATER COLUMN

Bulich, A. A. and D. L Isenberg. 1981. Use of the luminescent bacterial system for the rapid assessment of aquatic toxicity. ISA Transactions 20(1):29-33.

This article introduced the new Microtox luminescent bacterial test system (= marine bacterium, *Photobacterium phosphoreum* NRRL B-11177) designed to be a quick and sensitive bioassay tool. This article describes the basic operating system and provides a pictorial representation of the system. It also provides a synopsis of the responses of the Microtox system to pure compounds and effluents and compares these data to fish bioassays.

Methods:

Toxicity tests are conducted by adding reconstituted bacteria (the Microtox "reagent") to 2 ml test samples adjusted to 2% NaCl (to simulate the bacteria's native marine environment). The standard test conditions are: temperature = 15 °C, 5 min exposure time and the test endpoint = EC50 = point at which there is a 50% reduction in light emission. Test sensitivity can be increased by temperature adjustment over a range of 15-25 °C, increased exposure times (up to 15 min) and selection of different bacterial strains.

Results:

Comparative data are given for Microtox vs. fish assays for the following pure compounds and complex effluents:

Pure Compounds (mg/liter):

Toxicant	Microtox 5 Min EC50	Fish Assay 96 Hour LC50		
Mercury II	0.065	0.01	-	0.9
Pentachlorophenate	0.5	0.21	-	0.6
Aroclor 1242	0.7	0.3	-	1.0
p-Cresol	1.5	3.5	-	19
Sodium lauryl sulfate	1.6	5	-	46
Ammonia (free)	2.0	0.068	-	8.2
Benzene	2.0	17	-	32
Zinc II	2.5	0.24	-	7.2
Malathion	3.0	0.07	-	19.5
Formaldehyde	3.0	18	-	185
Copper II	8.0	0.1	-	10.7
Cyanide (HCN)	8.5	0.1	-	0.44
Trinitrotoluene	20		26	
Phenol	25	9	-	66
Chromium IV	70	29	-	133
Nitrate	420	19	-	230
1-Butanol	3300		1940	
Urea	24,000		12,000	
Ethanol	31,000		13,500	
Isopropanol	42,000	4,200	-	11,130

Complex Effluents (%):

Sample Number	Microtox 5 Min EC50	Fish Assay 24 Hour LC50
1	0.0322	0.1
2	0.2	0.07
3	1.3	0.5
4	1.6	21.7
5	2.6	2.4
6	4.3	29
7	13	12.2
8	15	68
9	21	18
10	22	42
11	24	14
12	25	32
13	62	52
14	68	NL
15	70	100
16	82	60
17	NE	75
18	NE	91
19	NE	NL
20	NE	NL

NE = No Effect

NL = No Lethality

The authors claim that Microtox is generally as sensitive to pure compounds and effluents as 96-hr fish bioassays.

Bulich, A. A, M. W. Greene and D. L. Isenberg. 1981. Reliability of the bacterial luminescence assay for determination of the toxicity of pure compounds and complex effluents. Pp. 338-347 *In: Aquatic Toxicology and Hazard Assessment: Fourth Conference, ASTM STP 737*, D. R. Branson and K. L. Dickson, eds. Am. Soc. for Testing and Materials, Philadelphia, PA.

This study determined the sensitivity of the newly developed Microtox assay system to a variety of pure compounds and compared these results to data from similar fish bioassays. Data were also collected for a variety of complex effluents using Microtox side-by-side with fish and invertebrate assays. Routine tests were also conducted with the reference toxicant sodium lauryl sulfate (SLS) to measure reproducibility of Microtox results.

Methods:

Microtox assays were 5-min exposures at 15 °C in a 2% NaCl matrix to a series of toxicant concentrations so that EC50s (light reduction relative to controls) could be calculated. Fish and

invertebrate tests used both static and flow-through, 24 to 96-hr exposures of rainbow trout, fathead minnow, bluegill, sheepshead minnow, *Daphnia* and mysids; no other experimental conditions for these bioassays were given.

Results:

Five-min EC50s for Microtox with pure compounds were as follows (all in mg/liter; fish data are from the published literature):

Toxicant	Microtox 5 min EC50	Fish Assay 24 to 96 hr LC50	
Mercury II	0.065	0.01 to	0.9
Pentachlorophenate	0.5	0.21 to	0.6
Aroclor 1242	0.7	0.3 to	1.0
<i>p</i> -Cresol	1.5	3.5 to	19
Sodium lauryl sulfate	1.6	5 to	46
Ammonia (free)	2.0	0.068 to	8.2
Benzene	2.0	17 to	32
Zinc II	2.5	0.24 to	7.2
Malathion	3.0	0.07 to	19.5
Formaldehyde	3.0	18 to	185
Copper II	8.0	0.1 to	10.7
Cyanide (HCN)	8.5	0.1 to	0.44
Trinitrotoluene	20		26
Phenol	25	9 to	66
Chromium VI	70	29 to	133
Nitrite	420	19 to	230
1-Butanol	3,300		1,940
Isopropanol	42,000	4,200 to	11,130
Urea	24,000		12,000
Ethanol	31,000		13,500

Generally, there were good agreements between Microtox EC50s and fish LC50s. There were also generally good agreements for the side-by-side effluent test results. Microtox effluent EC50s ranged from 0.032% to >100%.

For SLS tests, 81 EC50 determinations showed a mean value of 1.57 mg/liter with a CV of 18.2%.

Chang, J. C., P. B. Taylor and F. R. Leach. 1981. Use of the Microtox Assay System for environmental samples. Bull. Environ. Contam. Toxicol. 26:150-156.

The authors used the new Microtox Assay System to test the toxicity of various pure compounds, natural waters, pesticides and oil refinery effluents.

Methods:

Five-min EC50s were calculated for exposures to various compounds at 15 ± 0.1 °C in a 2% NaCl matrix.

Results:

Comparative Microtox EC50s, fish 96-hr LC50s and rat oral LD50s are given below:

Compound	Microtox EC50 (mg/liter)	Rat (oral) LD50 (g/kg)	Fish Toxicity LC50 (mg/liter)
Ethanol	47,000	14	13,000
1-Butanol	44,000	4.4	1,900
Benzene	200	5.7	50
Toluene	50	5.0	23
Phenol	26	0.53	5.0
<i>m</i> -Cresol	11	2.0	19 (<i>p</i> -Cresol)
Formaldehyde	8.7	0.80	250

Respiratory Inhibitors

Amytal	1,000
Thenoyltrifluoroacetone	3.5
Cyanide	2.5
Azide	400
Arsenate	94

Oil Refinery Effluents (%)(Fathead Minnow)

ETE - 55	58	65
17 - 51 - 80	74	65
LNX	100	65
UQB - 3	1.8	42
UQB - 4	100	75

Pesticides

Captafol	7	6,200
Carbaryl	2	500
Cyhexatin	10	540
Diazinon	1.7	300
Dichloran	3	5,000
DDT	7	110
Glyphosate	7.7	4,300
Malathion	10	1,400
Paraquat	780	150
Ridomil	120	670
Thiabendazole	3,400	3,100

Lebsack, M. E., A. D. Anderson, G. M. DeGraeve and H. L. Bergman. 1981. Comparison of bacterial luminescence and fish bioassay results for fossil-fuel processes waters and phenolic constituents. Pp. 348-356 *In: Aquatic Toxicology and Hazard Assessment*. Fourth Conference, ASTM STP 737, D. R. Branson and K. L. Dickson, eds. Am. Soc. for Testing and Materials, Philadelphia, PA.

The authors tested the toxicity of waste waters produced by a number of experimental oil shale retorts using Microtox and compared these results with rainbow trout and fathead minnow bioassays of similar waters.

Methods:

Oil shale retort process waters (Omega-9 water) were tested via Microtox using light diminution (EC50s) of *Photobacterium fischeri* as the endpoint. Tests were conducted at 15 °C in a 2% NaCl matrix with 5-min exposure times.

Results:

Rainbow trout were generally more sensitive and fathead minnows generally less sensitive to Omega-9 and similar process waters than Microtox. However, Microtox was generally less sensitive to phenolic compounds than either fish species, as shown in the following table (all concentrations in mg/liter):

Compound	Microtox EC50	Rainbow Trout LC50	Fathead Minnow LC50
Resorcinol	310	>100	100
Catechol	32	8.9	3.5
<i>o</i> -Cresol	32	8.4	18
Phenol	25	8.9	68
Benzonitrile	19	32	64
<i>m</i> -Cresol	8.2	8.9	56
<i>p</i> -Cresol	1.3	8.6	29
Hydroquine	0.079	0.097	0.044
Benzoquine	0.0085	0.13	0.045

Qureshi, A. A, K. W. Flood, S. R. Thompson, S. M. Janhurst, C. S. Inniss and D. A. Rokosh. 1982. Comparison of a luminescent bacterial test with other bioassays for determining toxicity of pure compounds and complex effluents. Pp. 179-195 *In: Aquatic Toxicology and Hazard Assessment: Fifth Conference*, ASTM STP 766, J. G. Pearson, R. B. Foster and W. E. Bishop, eds. Am. Soc. for Testing and Materials, Philadelphia, PA.

The authors conducted Microtox bioassays of single chemical compounds and complex effluents and compared the results with the results of rainbow trout, *Daphnia* and bacterial bioassays of the same toxicants.

Methods:

Microtox: Tests used a prototype model of the Microtox system with lyophilized *Photobacterium phosphoreum* in a 2% NaCl matrix. Test temperature = 15 ± 0.3 °C. Test endpoint = 5 min light decrease (=EC50) relative to controls.

Rainbow trout: (*Salmo gairdneri*): Tests were 96-hr static bioassays with aeration except for volatile compounds which were used with 24-hour exposures without aeration. Temperature = 15 ± 1.0 °C, pH = 7.8-8.1, fish = 0.5 to 3.0 g each (young-of-the-year), unfed, and the test endpoint = mortality (LC50s).

Daphnia magna: Tests used ≤ 24 -hr old animals in a static, unaerated test system with 100 ml test solution/tank, 15 ± 1.0 °C, 16 hr light/8 hr dark cycle, unfed, exposure time = 48 hrs, and test endpoint = mortality (48-hr LC50s).

Bacteria (*Spirillum volutans*): Tests were conducted in test tubes with 0.9 ml of solution at 20-22 °C. Drops of bacteria/toxicant solution were viewed at 100X under a compound microscope to assess "reversing motility." Test endpoint = minimum effective concentration necessary to eliminate motility in >90% of the cells (= MEC90s).

Results:

Generally, the bacterial assays (*Spirillum*) were least sensitive followed by *Daphnia*, Microtox, and fish. However, there was little agreement between the tests. Thus, it's important to use a battery of assays for a testing program.

Comparative results for single compounds are as follows (mg/liter):

Toxicant	Microtox 5 min EC50	Fish 96 hr LC50	Daphnia 48 hr EC	<i>Spirillum</i> 5 min MEC90
Copper (CuSO ₄)	7.4	0.25	0.02	7.4
Zinc (ZnSO ₄)	49.0	2.2	5.1	7.2
Mercury (HgCl ₂)	0.08	0.21	0.03	3.7
Arsenate (as As)	35.0	43	5.4	3,070
Cyanide (as free KCN)	13.3	0.15	6.1	1.7
Ammonia (total)	3,607	62	129	2,420
Ammonia (un-ionized)	1.5	1.4	0.8	0.7
Phenol	22.0	9.9	32	400
Styrene	5.4	2.5	59	636
Chloroform	435	32	758	2,460
1,2 Dichloroethane	158	198	1,430	4,060

Comparative results for the effluents are as follows (% volume/volume):

Effluent	Microtox 5 min EC50	Fish 96 hr LC50	Daphnia 48 hr EC50
Pulp Mills:			
PM-A	2.5	17	34
PM-B	8.4	37	NT*
Chemical Plants:			
CP-A	50 - 100	51	NT
CP-B	15	71	23
CP-C	40	7.1	NT
CP-D	34	NL**	39
Oil Refineries:			
OR-A1	6.5	71	78
OR-A2	50 - 100	NL	NT
Packaging Plant Dye Wastes:			
PP-A	1.5	0.9	0.3
Sewage Treatment Plant:			
STP-A1	>100	NL	NL
STP-A2	>100	NL	NL
STP-A3	30	43	16

* NT = not tested

** NL = non-lethal

Samak, Q. M. and R. Noiseux. 1980. Acute aquatic toxicity measurement by the Beckman Microtox. Summary of a paper presented at the Seventh Annual Aquatic Toxicity Workshop, Montreal, Canada, Nov. 1980. 17 pp.

The authors tested the toxicity of several pure compounds and several petrochemical effluents with Microtox. They also tested the toxicity of an effluent over a pH range of 5-9. Some comparisons are made with zebrafish (*Brachydanio rerio*) LC50s.

Methods:

Microtox tests were conducted as per the manufacturers recommendations (5-minute exposures, 15 °C and a 2% NaCl matrix). Zebrafish tests were static 72-hr exposures with moderate aeration.

Results:

Compound	Microtox Approximate EC50 (mg/liter)
Cyanide	6.5
Phenol	42
Sulphide	13.5
Naphthalene	2
Xylene	15
Toluene	48
Diethanolamine	72
Benzene	>100

For wastewater, the EC50s were fairly stable at a pH range from 5.5 to 8.0. The correlation coefficient between Microtox EC50s and zebrafish LC50s was 0.884.

SCCWRP. 1989. Toxicity of stormwater runoff in Los Angeles County. Pp. 66-71 *In: South. Calif. Coast. Water Res. Proj. Annual Report 1988-1989*, Long Beach, CA.

This study used the Microtox Toxicity Analyzer System to assess the toxicities of storm-generated runoff waters from the Los Angeles and San Gabriel Rivers and from Ballona Creek in Los Angeles County, California.

Methods:

Storm water runoff was collected \pm hourly from the rivers on four occasions. Samples were stored at 4 °C and analyzed within 2 days following settling or centrifugation. The test bacteria = *Photobacterium phosphoreum*, tested in a 2% NaCl matrix for 30 minute exposure times (test temperature was not given but was probably 15 °C). Test endpoint = decreased light output relative to the controls.

Results:

The mean toxicities of each river were: San Gabriel River = 18%, Ballona Creek = 31% and Los Angeles River = 13-45%. The range in toxicity for the Los Angeles River = 6-67%. Toxicities seemed to be highest during the first surge of a storm event and were generally greater during smaller runoff events (less dilution of toxicants??). Principal component and multiple regression analyses pointed to correlations of toxicity with suspended and volatile suspended solids. Comparisons of the runoff toxicity with sewage effluents from 4 plants showed that sewage effluent toxicities were greater than the runoff toxicities.

MISCELLANEOUS

Microbics Corporation. 1988. Microtox bibliography. Unpublished bibliographic listing of Microtox studies compiled by Microbics Corporation, 222 Rutherford Road, Carlsbad, California 92008. 6 pp.

Roughly 150 Microtox-related studies are referenced in this unannotated bibliography. It covers studies from 1974 to 1988 and each entry contains a Microbics library reference number.

CHAPTER 6. GEODUCK (*PANOPEA GENEROSA*) BIOASSAYS

SEDIMENTS

Chan, S., D. W. Brown, M. H. Schiewe and U. Varanasi. 1987. Seattle Harbor Operations and Maintenance Project: Physical/chemical/biological analyses of sediments proposed for operations and maintenance dredging. Final Rpt. by Environ. Conservation Div., NW & Alaska Fish. Center, NMFS/NOAA, Seattle, WA. 160 pp.

This study used amphipod, Microtox and juvenile geoduck (1.5-5 mm long) and sand dollar (2-15 mm diameter) assays to test Duwamish Waterway and Eagle Harbor sediments.

Methods:

For the geoduck tests, method used 2 cm sediments in the bottom of 400 ml cylinders, a flow-through system at 11-14 °C and fed fish meal.

Results:

Almost all test results showed juvenile geoducks to be insensitive based on the endpoints of survival, growth, tissue proteins, triglycerides and adenylate energy charge. The authors did not recommend geoducks for further use as a bioassay tool.

See this listing under "Multiple Bioassays" for methods and results of the other test organisms.

Chan, S. 1988. NOAA sublethal bioassay report. Pp. E-57+, Exhibit E.22 *In: PSDDA Reports: Evaluation Procedures Technical Appendix - Phase I (Central Puget Sound).* Seattle District, U.S. Army Corps of Engineers, Seattle, WA.

This is a letter report (which is incorporated as an exhibit in the above named PSDDA document) from NMFS/NOAA, Seattle, which summarizes the Final Report by Chan et. al. (May 1987) titled: Seattle Harbor Operations and Maintenance Project: Physical/chemical/biological analyses of sediments proposed for operations and maintenance dredging (see above).

Crecelius, E. A. 1985. Results of 10-day geoduck bioassays of Everett Harbor sediments. Letter Report to Washington Department of Natural Resources, Olympia, WA by Battelle Marine Research Laboratory, Sequim, WA. 2 pp.

Juvenile geoducks, 5-mm long, were exposed to 100 ml wet sediments in 800 ml seawater for 10 days at 15 °C with aeration in a static system. Geoducks were fed algae every other day.

Results (number of survivors out of 20):

Replicate Beaker #	Sequim Bay Control	Everett Composite "Clean"	Everett Composite "Dirty"
1	20	20	0
2	20	18	0
3	20	20	0
4	0	19	0
5	20	18	0

Side-by-side amphipod (*R. abronius*) exposures to the same sediments resulted in little toxicity to the amphipods.

Schiewe, M. and D. Misitano. 1986. Juvenile geoducks and sand dollars studied for use in long-term sediment bioassays. Pp. 17-19 In: Quarterly Report, July-Sept. 1986, NW & Alaska Fish. Center, NMFS/NOAA, Seattle, WA.

The authors conducted 30-day flow-through exposures of juvenile geoduck and juvenile sand dollars to sediments from the Duwamish Waterway and Eagle Harbor.

Results of sediment assays with amphipods (*R. abronius*) and Microtox showed that some of the sediments were acutely and sub-leathally toxic. For geoducks, there were no significant differences in survival, growth or tissue concentrations of total proteins or triglycerides for animals exposed to the test sediments. For sand dollars, significant differences in growth were observed in several of the Duwamish samples. The authors concluded that geoducks are not particularly sensitive to contaminated sediments.

CHAPTER 7. MULTIPLE TESTS

SEDIMENTS

Anderson, J. W. and E. A. Crecelius. 1986a. Physical, chemical and biological analyses of sediments proposed to be dredged for the Oak Harbor Marina Expansion Project, Oak Harbor, Whidbey Island, Washington. Final Rpt., Contract No. DE-AC06-76RL0 1830, for the U. S. Army Corps of Engineers, Seattle District by Battelle Marine Research Laboratory, Sequim, WA. 28 pp. + appendix.

The authors used amphipod, *Rhepoxynius abronius*, and Microtox bioassays to test toxicity of Oak Harbor sediments collected during Feb. 1985. Selected physical/chemical parameters were measured.

Methods:

For the amphipod tests, used the "Swartz protocol" (Swartz et al. 1985), 10-day exposures at 20 °C, 20 amphipods/replicate, 5 reps/sediment. Test endpoints = mortality and 1-hr reburial success at termination. Five test sediments + West Beach native control and Sequim Bay reference sediments were tested.

For Microtox, used organic extract following the method of Schiewe et al. (1985), 15-min EC50s for sediments stored for 5 months at 4 °C.

No data given on physical/chemical monitoring of the bioassays including temperature, pH, DO, salinity, amount of sediment used in the chambers, chamber type, aeration, etc for either assay.

Results:

Amphipod tests: Mean control survival (West Beach) = 90%. Sequim Bay Reference survival = 63%. Oak Harbor test sediment survivals ranged from 54% to 83%. No significant effects for reburial success observed. Amphipod mortalities had no obvious correlations with measured contaminant concentrations.

Microtox: Sequim Bay reference sediment was the most toxic with 15-min EC50s of 0.3 to 1.6 µl/ml. Test sediment EC50 range = 0.83 to 29.39 µl/ml. There was no relation between amphipod survival and the Microtox results.

Anderson, J. W. and E. A. Crecelius. 1986b. Collection and analyses of sediments from Kenmore Navigation Channel Project for chemical contamination and biological effects. Final Rpt., Contract No. DE-AC06-76RL0 1830, for the U. S. Army Corps of Engineers, Seattle District by Battelle Marine Laboratory, Sequim, WA. 15 pp.

The authors tested the toxicity of Lake Washington (freshwater) sediments with amphipods (*Rhepoxynius abronius*) and Microtox. They tested 7 samples + native control (West Beach) sand and Sequim Bay reference sediment.

Methods:

Amphipod assay: 10-day exposures via "Swartz protocol" (Swartz et al. 1985), 20 amphipods/chamber, 5 reps/sediment. Sediments held for 30 days in seawater at 15 °C prior to testing to adjust the interstitial salinities. No data given on amounts of sediment, temperatures, salinity, pH or DO.

Microtox: Used organic extracts with 15-min EC50s. No other data provided.

Results:

Amphipod mean survival in the West Beach control = 94%, Sequim Bay reference = 74%, and the range of survival for the Kenmore test sediments = 40-87%. Data on emergence and reburial were also given. Highest amphipod mortalities were associated with the cleanest samples. There was a possible grain size effect on survival.

For Microtox, Sequim Bay sediments were the most toxic with EC50s ranging from 0.3 to 1.6 µl/ml. Kenmore test sediment EC50s ranged from 5.3 to 17.1 µl/ml. "These toxicity results do not strongly correlate with either sediment chemistry or amphipod bioassay results."

Chan, S., D. W. Brown, M. H. Schiewe and U. Varanasi. 1987. Seattle Harbor Operations and Maintenance Project: Physical/chemical/biological analyses of sediments proposed for Operations and Maintenance dredging. Final Rpt. by the Environmental Conservation Division, NW and Alaska Fish Center, NMFS/NOAA, Seattle, WA. 160 pp.

The authors used amphipod, Microtox, juvenile geoduck and sand dollar assays to test Duwamish River and Eagle Harbor sediments.

Methods:

Amphipod: *Rhepoxynius abronius*, in 175 ml (2 cm) sediments at 15 °C for 10 days.

Microtox: EC50 determinations in terms of µl sediment/liter of solvent (organic or seawater?).

Geoduck: *Panopae generosa*, 1.5-5 mm long, in 2 cm sediment in 400 ml cylinder, flow-through system, 30-day exposures, fed algae.

Sand dollar: *Dendraster excentricus*, 2-15 mm diameter, 1 cm sediment in glass dish, flow-through system, 11-14 °C, fed fish meal.

Reference sediments: West Beach, Sequim Bay, Dabob Bay, Tolmie State Park.

Results:

Amphipods: 9 of 12 Duwamish sediments caused significant mortality. Survival in the reference sediments = 80-98% with some significant differences relative to West Beach sand.

Microtox: 10 of 12 Duwanish River sediments had EC50s <150 μ l/liter. All reference sediments were >500 μ l/liter except Sequim Bay = only 70 μ l/liter.

Geoduck: Almost all tests were insensitive based on survival, growth, tissue proteins, triglycerides and adenylate energy charge endpoints. **Not recommended for future bioassays.**

Sand dollars: Survival generally good in all sediments, but growth was reduced in some Duwamish River sediments. This species is proposed for further evaluation.

Chan, S., M. H. Schiewe, K. L. Grams, A. J. Friedman, R. G. Bogar, U. Varanasi, W. L. Reichert, P. D. Plesha, S. J. Demuth and D. W. Brown.
1986. East, West and Duwamish Waterways Navigation Improvement Project: Physical/chemical/biological analyses of sediments proposed for dredging. Final Rpt. by NW and Alaska Fish. Center, NMFS/NOAA, Seattle, WA. 106 pp.

Amphipod (*Rhepoxynius abronius*) and Microtox tests were used to assay 30 sediment samples from the Duwamish River. They also conducted bioaccumulation tests with the clam, *Macoma nasuta*. Amphipod = 10-day "Swartz" test in 175 ml (2 cm) sediments in 1 liter glass beakers at 15 °C and 28-30 % salinity. Microtox = 15-min EC50s with organic extracts.

Results:

Amphipods: Mean survival in West Beach sand = 95-99%, Sequim Bay reference = 83-96%, and Duwamish River = 18-95%. 19 of 30 sediments had significantly reduced survival when compared to West Beach. 12 of 30 had significantly reduced survival when compared to Sequim Bay reference. 7 of 30 had significantly reduced survival when compared to Four Mile Rock (Elliott Bay) disposal site sediments.

Microtox: Duwamish River 15-min EC50 range = 32.3-8,880 μ l/liter. Sequim Bay reference = 69.5 μ l/liter (one of most toxic). 17 of 19 sediments with EC50s <500 μ l/liter failed chemistry and amphipod tests. DDT, PCB and HMW AHs bioaccumulated in *Macoma* from 5 Duwamish River test sediments.

Of the 30 sediments tested, 18 failed chemical criteria for disposal and 6 of those 18 also failed the amphipod mortality criterion. Microtox responses had a highly significant association between EC50s and concentrations of AHs and CHs.

Chapman, P. M., R. N. Dexter, R. D. Kathman and G. A. Erickson. 1984a. Survey of biological effects of toxicants upon Puget Sound biota. IV. Interrelationships of infauna, sediment bioassay and sediment chemistry data. NOAA Tech. Memo. NOS OMA 9. 57 pp.

This report pulls together data from three sediment studies conducted in Puget Sound by NOAA/NMFS, METRO and EPA. All studies collected data on chemical concentrations, benthic infauna and bioassays. Various correlations and indices are discussed using the combined data

sets including the Index of Benthic Degradation (IBD) and the Sediment Triad Approach. Literature citations for the individual studies are:

NMFS = Chapman et al. 1982; Chapman et al. 1984; Malins et al. 1980.

METRO = Comiskey et al. 1983

EPA = Swartz et al. 1982

Bioassay data included in the discussion include: Amphipod mortality, oligochaete respiration, oyster larvae abnormality, fish cell effects, polychaete life-cycle effects and surf smelt partial life-cycle effects.

General Findings:

- 1) Echinoderms and crustaceans (esp. *Rhepoxynius* spp.) were absent from degraded areas. These areas were dominated by polychaetes and molluscs.
- 2) "Stations and areas of Puget Sound with higher levels of chemical contamination and sediment toxicity contain benthic communities indicative of environmental degradation."
- 3) "Elevated sediment levels and positive toxicity test results correspond with, and are possibly indicative of, actual effects on benthic communities."
- 4) The Index of Benthic Degradation and the Triad Approach proved to be functional tools for defining degraded areas.
- 5) All three urban bays (Commencement Bay, Elliott Bay and Sinclair Inlet) proved to be substantially degraded compared to reference areas (Case Inlet and Samish Bay—Samish Bay appeared to be the best reference area of the two).

See the individual studies cited above for more specific details of each study.

Chapman, P. M., R. N. Dexter, J. Morgan, R. Fink, D. Mitchell, R. M. Kocan and M. L. Landolt. 1984b. Survey of biological effects of toxicants upon Puget Sound biota - III. Tests in Everett Harbor, Samish and Bellingham Bays. NOAA Tech. Memo. NOS OMS 2, Rockville, Maryland. 48 pp.

This study used amphipod, oyster embryo, oligochaete respiration and fish cell culture bioassays to measure toxicities of 23 sediment samples from Everett Harbor, Bellingham Bay and Samish Bay (reference area). Data were also provided on grain sizes and chemical contamination (analyzed as part of this study, but the results are reported elsewhere). Sediments were collected in May 1983 with a van Veen grab. Seven to ten subcores were taken from each grab and composited. Sediments for the bioassays were frozen prior to testing.

Methods:

Oyster embryo (*Crassostrea gigas*): 48-hr exposures to 15 g sediment in 750 ml UV-treated seawater in polyethylene bottles. 35 embryos/ml with 2 reps/sample. Sediments were mixed for 3 hrs prior to embryo addition, no agitation during testing. Embryos were filtered with 38 μ m Nytex screen at test termination. Survival and abnormality endpoints. Salinity = 25 %o, DO = 8 mg/liter and pH = 8.0 (all adjusted at T0).

Amphipod (*Rhepoxynius abronius*): 10-day exposures to 200 g solid phase in 800 ml seawater at 25 ‰ salinity, test temp. = 10.0 ± 0.5 °C, 12 hr light/dark cycle, with aeration. 20 amphipods/glass jar, 5 reps/sample. Test endpoints = survival and daily avoidance data.

Oligochaete (*Monopylephorus cuticulatus*) respiration: Worms were exposed for 3-5 hrs to saline elutriates prepared by adding 10 g sediment to 500 ml seawater and shaking for 1 min. Elutriates were tested at 25 ‰ salinity and 10.0 ± 0.5 °C. Response measured = respiration rates in $\mu\text{l O}_2$. Replication = 6 to 10X.

Fish cell culture reproduction and genotoxicity: Used 96-hr exposures of fish cell cultures to organic extracts. Used rainbow trout gonad cells (RTG-2) with mixed-function oxidase (MFO) activity and bluegill fry cells (BF-2) without MFO activity. Extracted 150 g sediment with various solvents and used a dilution series of 50, 25, 10, 5, 2 and 1 $\mu\text{g/ml}$. Test endpoints = cell numbers and anaphase aberrations.

Results:

Oyster embryo: End of test ranges for salinity = 25.0 to 25.2 ‰, pH = 7.4 to 7.9, DO = 4.6 to 6.9 mg/liter. Mean control (seawater) abnormal = 2.2% and 1.6% for West Beach sand. No initial counts of embryo densities were taken, thus seawater control survival was set at 100% at the end of the test. Relative survival in the control sediment = 92%. Seven test sediments were highly toxic, 7 of moderate toxicity, 5 of low toxicity and 3 were non-toxic. No toxicity was observed in the Samish Bay reference sediments.

Amphipods: Mean control and Samish Bay reference survivals all >90%. Only 1 station each in Bellingham Bay and Everett Harbor caused significant mortality. The avoidance results were variable and of little use.

Oligochaete respiration: Sediments from 7 of 22 stations produced sublethal stress.

Fish cell culture: For cell reproduction, 8 sediments significantly reduced cell growth for RTG-2 cells and 3 sediments reduced growth for the BF-2 cells (only one was common to both cultures). The 2 Samish Bay reference sediments showed significant reductions in cell numbers. For genotoxicity, 8 of 22 sediments showed significantly increased anaphase aberrations (RTG-2 cells tested only) including 1 Samish Bay reference sediment.

Generally, Bellingham and Everett Harbor sediments were less toxic than other areas (e.g., Duwamish Waterway and Commencement Bay), with Everett more toxic than Bellingham Bay sediments.

Chapman, P. M., R. N. Dexter, R. M. Kocan and E. R. Long. 1985. An overview of biological effects testing in Puget Sound, Washington: Methods, results, implications. Pp. 344-363 *In: Aquatic Toxicology and Hazard Assessment: Seventh Symposium*, R. D. Cardwell, R. Purdy and R. C. Banner, eds. ASTM STP 854, Am. Soc. for Testing and Materials, Philadelphia, PA.

This article describes results of testing 97 stations (Phase I) and 22 stations (Phase II = a subset of Phase I stations) for sediment toxicity using 7 different test systems:

- 1) Amphipod, oligochaete and stickleback lethality at 20,000 ppm sediment, 10 °C and 25 ‰ salinity.
- 2) Oligochaete respiration at 20,000 ppm sediment elutriate, 10 °C and 25 ‰ salinity.
- 3) Mitoses in fish cell culture with organic extracts.
- 4) Oyster larvae assay with solid phase, 48-hr, 20 °C and 25 ‰ salinity.
- 5) Lethal and sublethal effects on surf smelt eggs and larvae with solid phase, 10-day exposures, 14 °C and 11.5 ‰ salinity.
- 6) Lethal and sublethal effects on a polychaete life cycle with solid and elutriate phases.
- 7) *In vivo* fish cell reproduction/genotoxicity tests with organic extracts.

Information presented in this paper is an overview of data presented in detail in Chapman et al. 1982, NOAA Tech. Memo OMPA-25 and Chapman et al. 1983, NOAA Tech. Rpt. NOS-102-OMS-1.

Results:

- 1) No mortality was observed in the amphipod (*Eogammarus confervicolus*), oligochaete or stickleback tests in any samples except 40% mortality of amphipods in a single Elliott Bay sample.
- 2) Worm respiration showed significant differences in 40 of 97 sediments with some depressed (44%) and some elevated (56%).
- 3) Genotoxicity testing (rainbow trout gonad cell culture) showed toxic responses (inhibition of growth) in 30 of 97 stations and anaphase aberrations in 58 of 97 stations.
- 4) For oyster larvae, 12 of 22 sediments showed >20% larval abnormality with the same general trend for mortality.
- 5) Great deal of variability for surf smelt tests.
- 6) *Capitella capitata* life-cycle tests showed that larval development/settlement was most sensitive stage.

Chapman, P. M., R. N. Dexter and E. R. Long. 1987. Synoptic measures of sediment contamination, toxicity and infaunal community composition (the Sediment Quality Triad) in San Francisco Bay. *Marine Ecol. Progress Series* 37:75-96.

Sediments from 3 stations in each of 3 areas of San Francisco Bay were tested for chemicals, toxicity and benthic infauna (testing triad). Sites = San Pablo Bay (reference area), Oakland Bay and Islais Waterway. Bioassays used were:

Amphipod (*Rhepoxynius abronius*): 10-day survival and avoidance endpoints, temp. = 14.5-16.6 °C, salinity 27-30 ‰, pH 7.9-8.4, DO > 5.0 mg/liter.

Mussel (*Mytilus edulis*): 48-hr larval survival/abnormality assay. Method of Mitchell et al. 1985. Temp. = 18-20.5 °C, salinity 27-28 ‰, pH 8.1-8.4, DO 4.8-7.0.

Clam (*Macoma balthica*): 48-hr reburial test. Temp. = 15.0-16.5 °C, salinity 27-31 ‰, pH 8.0-8.5, DO >4.7 except for one at 3.6 mg/liter.

Harpacticoid copepod (*Tigriopus californicus*): Reproductive success test. 4-wk test at temp. = 17-20 °C, salinity 30-35 ‰, pH 7.8-8.5, DO >4.0 mg/liter.

Results:

Amphipod test: Two stations at Islais Waterway had significant decreases in survival. No significant differences in the avoidance tests.

Mussel larvae: For % abnormal, 2 Oakland Bay and all Islais Waterway sediments showed significant increases in abnormal over the sediment control. For % survival, all except one San Pablo Bay sediment showed significant increases in mortality. The sediment control mortality was significantly increased over the seawater control. Seawater control = 100% vs. the sediment control = 73.4 %. NOTE: Absolute seawater control mortality was not counted.

Clam reburial: Only two Islais Waterway stations had significantly increased reburial times.

Copepod reproduction: No significant effects on survival. Three Islais Waterway and 2 San Pablo Bay sediments had significant decreases in the number of young produced.

All three measures (chemistry/toxicity/infauna) showed that relative contamination was Islais Waterway > Oakland Bay > San Pablo Bay. In the most contaminated site (Islais), polychaetes dominated the infauna and amphipods were almost absent.

Ratio to Reference (San Pablo Bay) Values:

Mean Values	San Pablo Bay (control)	Oakland Bay	Islais
Chemistry (aggregate)	1.00	1.97	6.29
Bioassay (aggregate)	1.0	1.3	3.1

CH₂M Hill. 1989. Remedial Investigation, Eagle Harbor Site, Kitsap County, Washington. Data Reports, Volume 1, Parts 1-3. Final Rpt., Contract No. 68-01-7251, for the U. S. Environmental Protection Agency by CH₂M Hill, Bellevue, WA.

This study used amphipod (*Rhepoxynius abronius*) and Pacific oyster (*Crassostrea gigas*) larval bioassays to test sediments collected from Eagle Harbor, Bainbridge Island, WA. Reference sediments were collected from Port Madison and Yukon Harbor (sites north and south of Eagle Harbor). Control sediments and seawater were from Yaquina Bay, OR. 68 Eagle Harbor sediment samples were tested, but many of the samples exceeded the maximum holding time (2 weeks - PSEP Protocol) by 1-7 days. Test samples were collected in June 1988.

Methods:

Amphipod tests: Generally followed the PSEP Protocol. 10-day static exposures, temp. = 14-16 °C, pH 7.6-8.4, DO >6 mg/liter. Interstitial salinities were < 25 ‰ in 12 of the samples (as low as 15.4 ‰).

Oyster embryo tests: Generally followed PSEP protocol with 48-hr exposures. All samples exceeded the specified test temperature range of 19-21 °C by up to 1.7 °C. Salinities were outside of the specified 27-29 ‰ range in 75% of the samples (outside this range by up to 1.1 ‰).

pH = 7.5-8.0, DOs were less than the specified 5.0 mg/liter in 11% of the samples (with the lowest at 4.3 mg/liter).

Results:

Amphipods: Mean control survival was >90%. For Test #1, mean control survival = 99.2%. Test #2 control survival = 96.3%. Several of the Port Madison reference sediments showed substantial (>50%) mortalities. The range of survivals in the Eagle Harbor test sediments = 0.0 to 99.6%.

Oyster embryos: The mis-identification of abnormal embryos affected the Test #1 results. Test #1 seawater control mortality was <30%, control abnormal was <10%. Test #2 seawater control mortality = 15.4%, control abnormal = 12.4%. Thus, the control abnormal measure in Test #2 exceeded the PSEP Protocol specifications of ≤10%.

Test #1, Yaquina sediment control: Relative mortality = 31%, relative abnormal <10%.

Test #2, Yaquina sediment control: Relative mortality = 49.6%, relative abnormal = 7.8%.

There was a wide range of responses to the Eagle Harbor test sediments.

Note: These bioassays were subcontracted to NW Aquatic Sciences, Newport, OR. QA/QC review of the work was by PTI Consultants, Seattle, WA.

Dinnel, P. A., F. S. Ott and Q. J. Stober. 1984. Marine toxicology. Vol. X, Section 12 *In: Renton Sewage Treatment Plant Project: Seahurst Baseline Study*, Q. J. Stober and K. K. Chew, eds. Final Rpt. for the Municipality of Metropolitan Seattle (METRO) by the Fish. Res. Institute, Univ. of Wash., Seattle, WA. FRI-UW-8413:192 pp.

This study was one of about 10 related studies designed to provide baseline data for south central Puget Sound prior to possible installation of a deep-discharge sewage effluent outfall off Seahurst from the Renton Treatment Plant. This outfall was subsequently installed off Duwamish Head instead.

This section of work utilized sea urchin sperm and oyster embryo bioassays to monitor ambient water quality. These same assays + Dungeness crab larval assays were used to test the toxicity of Renton sewage (5 treatment stages). Amphipod assays of field-collected sediments were also conducted. Ancillary tests also investigated the toxicities of ammonia and phytoplankton metabolites using several of the above tests.

Methods:

Tests were conducted over a two-year period from 1982 to 1984. Water samples were tested several times/month during summer and fall (a few tests during winter) with oyster embryo and sea urchin/sand dollar sperm assays. Renton sewage was tested 10 times each during one summer and one winter period. Amphipod assays of sediments from south central Puget Sound were conducted 4 times each of 2 years.

Oyster embryo assays: 25,000-35,000 *Crassostrea gigas* fertilized eggs were exposed for 48 hours to seawater samples in 1 liter polypropylene beakers at 20 °C. Natural spawners were

used during the summer and oysters conditioned for 4-6 weeks during the winter. Test endpoints = survival and normal development to the "D"-shaped veliger stage.

Sand dollar (*Dendraster excentricus*) and green sea urchin (*Strongylocentrotus droebachiensis*) sperm assays: Methodology was that of Dinnel (1984). Generally, sperm were exposed for 60 min in 10 ml volumes prior to addition of the eggs for an additional 20 min fertilization period. Temperature = 13 ± 1 °C, sperm:egg ratio = 1,000:1 and the test endpoint = fertilization success.

Dungeness crab zoea: *Cancer magister* 1st zoea were exposed for 48 hrs in polypropylene beakers. Test endpoint = death.

Amphipod, *Rhepoxynius abronius*: Solid phase tests of Seahurst area sediments were conducted in both flow-through and static exposure systems. The flow-through system exposed 20 amphipods to sediments in Nytex mesh/PVC chambers with replicate (4-5) chambers contained in 15 liter aquaria provided with 500 ml/min of flowing ambient seawater. Amphipods and control sediments were from West Beach or Bowman Bay, Whidbey Island, WA. Sediment exposures = 10 days with 150 ml of sediment/chamber, chambers set on glass rods (to allow water circulation), constant light and no feeding. The static tests = protocol of Swartz et al. (1985) except that the beakers were polypropylene. Test endpoints = death and post-exposure reburial success. Note: all sediments were frozen prior to testing.

Results:

Toxicities observed in the ambient water samples were generally low to moderate and quite variable between dates and test type. Highest toxicity was in surface and bottom waters. There was a significant ($p < 0.01$) correlation between oyster embryo mortalities and chlorophyll "a" and pH. No substantial changes in toxicity were apparent compared to tests conducted by WDF from 1962 to 1976 for this same area. Note: About 60% of the oyster embryo assays failed the ASTM 30% criterion for control survival. However, only 18% failed a 50% criterion used for this study.

Relative toxicity of the Renton sewage was: chlorinated secondary > influent > primary > dechlorinated secondary > unchlorinated secondary. Relative sensitivity of the assays to sewage was: sperm assays > embryo abnormality > embryo mortality > crab zoea mortality. A 100:1 dilution of Renton sewage (projected outfall dilution) should prove non-acute toxic in Puget Sound with the possible exception of chlorinated effluent. Secondary treatment was very effective in removing a large degree of the toxicity of primary sewage.

For sediment assays, reduced amphipod survivals occurred in sediments primarily from the northern Seahurst stations and was correlated with both toxicant loads and grain size. Survivals in the static exposures were slightly less than for flow-through exposures.

Ecological Analysts, Inc. 1981. A technical evaluation of potential environmental impacts of proposed ocean disposal of dredged material at Winchester Bay, Oregon. Final Rpt. for the Portland District, U.S. Army Corps of Engineers by Ecological Analysts, Inc., Concord, CA.

Liquid-phase, suspended-phase and solid-phase sediment bioassays were conducted on sediments from Winchester Bay, Oregon. Reference sediment = offshore from near the ocean disposal site. Five stations were sampled in the bay but sediments from all stations were com-

posed into 1 test sample. Samples were collected with a Ponar grab during April and May 1981. Sediments were stored at 4 °C until testing.

Methods:

For the suspended-phase, mixed 1:4 ratio of sediment:seawater for 30 min (with aeration), settled 1 hr, and used supernatant for testing. For the liquid-phase, prepared as above except that the supernatant was filtered at 0.45 µm. For the solid-phase, used 20 mm test sediment overlaying 40 mm reference sediment. The solid phase was also used for bioaccumulation testing.

The test animals for the suspended and liquid-phase tests = Juvenile English sole (*Parophrys vetulus*), 19-75 mm in length; sand shrimp (*Crangon franciscorum*); and copepods (*Calanus pacificus*). For the solid-phase tests = Lugworm (*Abarenicola pacifica*); clam (*Macoma inequivalvis*); and amphipod (*Rhepoxynius epistomus*). Lugworms were also used for the bioaccumulation exposures.

The suspended and liquid-phase tests were static, 96-hr mortality tests in jars or aquaria. Used 5 reps/sediment, 7-10 organisms/chamber with 100, 50 and 10% dilutions.

Results:

Physical/chemical monitoring data (ranges):

	Temperature (°C)	DO (mg/liter)	pH	Salinity (‰)
Liquid and Suspended phases	11.0 - 14.6	5.7 - 11.1	7.5 - 8.1	NM*
Solid phase	11.5 - 17.8	2.3 - 11.1	6.0 - 8.2	NM

*NM = Not Measured

Control (reference sediment) survivals in all tests were >90% except for 85% for the copepods. Poor survivals (53-80%) in an artificial seawater control for all 3 liquid-phase animals. Significantly reduced survival only for the copepods in the liquid and suspended-phase test solutions and only for amphipods (69%, n=20 reps) in the solid phase test sediment. There may possibly have been a grain size and/or organics effect on the amphipods. No significant bioaccumulation of chemicals observed in the lugworms.

Galvin, D. V., G. P. Romberg, D. R. Houck and J. H. Lesniak. 1984. Toxicant pretreatment planning study summary report. METRO Toxicant Program Report No. 3, Water Quality Division, METRO, Seattle, WA. Chapter 5, pp. 122-137.

This is a summary report on METRO's Toxicant Pretreatment Planning Study (TPPS) written by METRO staff. Chapter 5 provides a summary of the biological effects sections of the study, including benthic infaunal community analyses, sediment bioassays and fish/shellfish tissue concentrations of toxicants. Full test details can be found in METRO's TPPS Technical Report C2.

The summary of bioassays includes a discussion of the amphipod (*Rhepoxynius abronius*) and oligochaete respiration assays. Irregularities with a chromosomal test precluded any discussion of these test results.

Generally, the test results with both amphipod and oligochaete respiration were poor; they were not consistent through time at the same stations, had little correspondence with priority pollutant concentrations or the benthic infaunal analyses. One exception was that responses were consistently recorded for bioassays off of the Denny Way CSO in Elliott Bay. Extra "dilution tests" designed to test the dose-responsiveness of amphipod assays failed due to low toxicity of the undiluted contaminated sediments. See TPPS Technical Report C2 for the details of methodology and results.

Hart Crowser, Inc. 1988. Results of sediment testing, Everett Marina, Everett, Washington. Final Report J-1661-04 for the Port of Everett by Hart Crowser, Inc., Seattle, WA. 8 pp. + appendices.

This project tested samples of sediments from the Everett Marina following PSDDA guidelines and the Puget Sound Estuary Protocols for bioassays. All samples were also analyzed for priority chemicals of concern. Grain size, conventional and biological toxicity testing using amphipod, oyster embryo and Microtox (saline extract) bioassays were measured.

Methods:

Used Puget Sound Estuary Program protocols for all bioassays. Bioassays included 10-day amphipod (*Rhepoxynius abronius*) assay; 48-hour oyster (*Crassostrea gigas*) larvae assay; and the saline extract Microtox assay. Bioassays were conducted by EVS Consultants, Vancouver, B.C.

Results:

Some chemicals exceeded PSDDA screening levels (SL's) in all five samples but none exceeded the maximum levels (ML's) in any sample.

Amphipod assay: Four of the five sediments produced significant ($p=0.05$) mortality as compared to West Beach control sediment, but none exceeded 30% mortality above control responses (controls = 96-98% survivals). The test salinities were 1-2 % lower than specified by the PSEP protocol.

Oyster embryo assay: One of five samples exhibited significant ($p=0.05$) abnormality (7.8%) and high (non-significant) mortality (83%) as compared to the seawater controls. However, seawater control mortalities (32.6 and 33.9%) exceeded the PSEP protocol standard of $\leq 30\%$. Starting salinity (26 %) was also slightly low relative to the PSEP protocol ($28 \pm 1\%$).

Microtox: The saline extracts showed no signs of toxicity.

Conclusion:

The one sample (DC-4) that showed significant toxicity in both the amphipod and oyster embryo assays is probably not suitable for unconfined open-water disposal in Puget Sound.

Johns, D. M. 1988. Puget Sound Dredged Disposal Analysis: Sublethal test demonstration study. Final Rpt. (Contract No. C728) for the U. S. Army Corps of Engineers, Seattle District, Seattle, WA by PTI Environmental Services, Bellevue, WA. 58 pp.

The author conducted sets of tests on sediments (and dilutions of those sediments) from Commencement Bay, Elliott Bay and Eagle Harbor (plus reference and control sediments) using a variety of organisms and test endpoints.

Specific tests and conditions:

Juvenile polychaete (*Neanthes arenaceodentata*): 20-day survival and biomass test. Static-renewal system, 20 °C, fed prawn flakes every 2nd day, salinity 28 ‰, DO >6.4 mg/liter, pH 7.5-8.5.

Amphipod (*Ampelisca abdita*): 14-20 day survival and biomass test. Flow-through system, 20 °C, fed algae 6 days/wk, salinity 28 ‰, DOs and pHs not given. Also, reproduction test with 28-day exposure, endpoints = number of juveniles produced, number of females recovered and female reproduction state.

Geoduck (*Panopea generosa*): Survival and burrowing activity endpoints. 10-day exposures in static-renewal or flow-through systems at 17-20 °C, fed every 2nd day, salinity 28 ‰, DO 7.0-8.3 mg/liter, pH 8.0-8.4.

Results:

***Neanthes*:** Very low 20-day mortality in all sediments; this was not a sensitive index. However, the 20-day growth (biomass) endpoint was rather sensitive and dose-responsive to all test sediments. The food ration can be a major variable affecting growth, but individual worm variability was not significant.

Amphipod: High shipping mortalities (collected in Rhode Island) resulted in very low survival in the control and test sediments in all runs. Thus, no usable results.

Geoduck: High survival was observed in all sediments, hence poor sensitivity to contaminated sediments. Also, high control mortalities in one test, probably due to shipping stresses. Burrowing was inhibited in some samples, esp. Eagle Harbor sediments. Year-round availability of standard-sized geoducks is questionable.

The author evaluated six objectives regarding test sensitivities, feasibility and cost. He concluded that the *Neanthes* 20-day growth test had the most promise for use as a PSDDA bioassay tool.

Long, E. R., M. F. Buchman, S. M. Bay, R. J. Breteler, R. S. Carr, P. M. Chapman, J. E. Hose, A. L. Lissner, J. Scott and D. A. Wolfe. 1989. Comparative evaluation of five toxicity tests with sediments from San Francisco Bay and Tomales Bay, California. Draft manuscript submitted to the Am. Chem. Soc. for publication in a book on "Biomarkers."

This study measured the biological effects of contaminated sediments from San Francisco Bay to provide supporting environmental quality data for NOAA's National Status and Trends Program. Fifteen sediments were tested with five organisms (multiple endpoints in each case) to define their relative sensitivity, analytical precision, discriminatory power and concordance with sediment chemistry. Test exposures included solid phase, elutriates and pore water.

Methods:

Sediments: Three samples each were collected from Oakland Harbor (= high contamination), Yerba Buena Island, San Pablo Bay, Vallejo (= low to moderate contamination) and Tomales Bay (= non-contaminated). Sediments were collected in Feb 1987 with a Young grab sampler. The upper 1 cm of replicate grabs was collected, homogenized and divided between bioassays and chemical/physical testing. Bioassays were conducted within 5 days of sediment collection.

Amphipod (*Rhepoxynius abronius*) solid phase tests: Used protocol of Swartz et al. (1985) with animals and control sediments collected from West Beach, WA. Temperature = 15 ± 1 °C, salinity = 28 ‰, 10 day static exposures, 2 cm sediment in 1 liter glass beakers, constant aeration and light, no feeding, 20 amphipods/beaker with 5 reps/sediment. Test endpoints = mortality, emergence from sediments and post-exposure reburial.

Amphipod (*Ampelisca abdita*) solid phase tests: This is a tube-forming amphipod collected from Buzzards Bay, MA. Testing used 200 ml (4 cm depth) of sediments in 1 liter Mason jars with an intermittent (18 turnovers/day) flow-through system with aeration. Exposure time = 10 days with 20 amphipods/beaker, 5 reps/sediment, no feeding, temperature = 20 ± 1 °C, salinity = 31-34 ‰, DO = 6.2-8.2 mg/liter and pH = 7.3-8.3. Some tests were also conducted with a static system for comparison. Test endpoints = mortality and emergence.

Mussel (*Mytilus edulis*) embryo solid/elutriate phase tests: Adults were collected from British Columbia and conditioned to spawn in the lab for 4 weeks at 14 ± 1 °C and fed algae. "Elutriates" were prepared by mixing 1:50 w/v of sediment/water for 10 seconds and allowing to settle 1 hour. The sediments were left in the beakers during testing. 15,000 fertilized eggs were added to 1 liter bottles, incubated 48 hrs at 17 ± 0.5 °C, 30 ‰ salinity, pH = 7.8, DO = 8.2 mg/liter (initial, no aeration) and a 14 hr light:10 hr dark photoperiod. Test endpoints = survival and normal development to a "D"-shaped veliger

Purple sea urchin (*Strongylocentrotus purpuratus*) sperm and embryo tests with elutriates: The Dinnel et al. (1987) protocol was used for the sperm/fertilization assays of elutriates (prepared with 1:4 v/v sediment/water mixture stirred overnight, settled 60 min and centrifuged at 2,000 G for 5 min) using 60 min sperm exposure times in 10 ml of solution. Test endpoint = egg fertilization success.

The embryo development assays used the protocol of Oshida et al. (1981). 7,500 fertilized eggs were added to 220 ml samples of elutriate (prepared as for the sperm assays) and exposed 48 hrs at 17 °C. Test endpoints = normal development to pluteus, echinochrome pigment synthesis and cytologic/cytogenetic abnormalities including number of mitoses, presence of micronucleated cells and mitotic (anaphase) aberrations.

Polychaete (*Dinophilus gyrocoiliatus*) pore water exposure tests: Pore water was extracted from the sediments with a plunger/cylinder system pressurized with compressed air.

Pore water was frozen until use. 1-2 day old worms were exposed up to 7 days to 10 ml of pore water in 20 ml stender dishes. Temperature = 20 ± 1 °C, salinity = 25 ± 1 ‰, pH = 8.0 ± 0.2 , DO = $\geq 80\%$ saturation, ammonia < 2 mg/liter, 4 animals/dish and food = 50µl of 0.5% suspension of spinach. Test endpoints = survival and egg production.

Supporting physical and chemical measurements included: grain size, TOC, TIC, metals and selected organic compounds.

Results (quoted from the manuscript):

"Among the endpoints evaluated, abnormal development of *M. edulis* embryos was the most sensitive to the 15 samples relative to controls and had the highest precision and discriminatory power. Survival of *R. abronius* was the second most sensitive and also had a high range in response and discriminatory power. The results of both end-points (along with those of *M. edulis* survival), however, were more highly correlated with sedimentological variables than with the concentrations of chemical contaminants. The end-point of *A. abdita* survival had relatively high analytical precision, moderate discriminatory power and was relatively highly correlated with several chemicals, but had relatively low sensitivity relative to controls. Abnormal development and echinochrome content in *S. purpuratus* had relatively high precision and results were relatively highly correlated with several chemicals, but discriminatory power was moderate and the abnormal development results contradicted those of many of the other end-points. Several of the cytological/cytogenetic end-points of this test (measured in only five samples) indicated a wide range in response and strong correlations with chemical data, but precision was relatively low. The test of *D. gyrocoelius* egg production was intermediate in sensitivity, had relatively low precision and discriminatory power, and was highly correlated with several organic chemical groups. The results of this pore water test were not highly correlated with those of the solid phase and elutriate tests. Three groups of the toxicity end-points indicated relatively high concordance with each other and with some of the same physical and chemical properties of the sediments. Others indicated relatively low concordance with each other and/or relatively high correlations with sedimentological variables, such as texture, compared to chemical toxicants. Since different toxicological mechanisms may occur in the responses of organisms to complex media such as sediments, additional research and evaluation of the individual tests is needed to further define their applicability. Also, multiple toxicity tests are needed to comprehensively assess the quality of marine sediments."

Malins, D. C., S. Chan, U. Varanasi, M. H. Schiewe, J. E. Stein, D. W. Brown, M. M. Krahn and B. B. McCain. 1984 (?). Bioavailability and toxicity of sediment-associated chemical contaminants to marine biota. Evaluation of short-term sediment bioassays: Final report on activities 1 and 4. NOAA/NMFS Project Rpt., Seattle, WA. 16+ pp.

The authors tested the sensitivity of six short-term bioassays to both field-collected sediments and sediments spiked with organic chemicals.

Bioassays tested:

Microtox: Tested organic extracts of 100 g of sediment, 15-min EC50s at 15 °C. Also took 5 and 30-min readings.

Amphipod (*Rhepoxynius abronius*): 10-day exposure test with 50 ml sediment + 900 ml seawater at 15 °C under constant light.

Oyster embryo (*Crassostrea gigas*): Used Chapman and Morgan method (1983), 20 g sediment mixed with 1 liter seawater for 5 min, 48-hr exposures at 20 °C.

Larval surf smelt: Used suspended particulate phase exposure, 20 g sediment + 1 liter seawater, mixed 5 min, settled 1 hr and used supernatants for testing. 60-75 larvae exposed in 900 ml supernatant at 9-12 °C for 7 days.

Copepod reproduction (*Tigriopus californicus*): Animals exposed to sieved particles (<64 µm). Three tests: 54 g sediment/500 ml seawater; 12 g/100 ml seawater; and 12 g/100 ml seawater. Exposed up to 4 wks at 18±1 °C, salinity 26-28‰. Test endpoint = Nauplii production.

Sea urchin fertilization (*Strongylocentrotus purpuratus*): Eggs and sperm added to the test solutions at the same time (no pre-exposures of the sperm), 15-min test at 9 °C.

Results:

There was significant toxicity of natural sediments from Eagle Harbor and the Duwamish Waterway (as compared to Dosewallips reference sediments) for the amphipod, oyster embryo and surf smelt larvae tests. No significant toxicity was observed in sediments from Useless Bay, Port Madison and West Point. Everett Harbor was toxic only to amphipods. The oyster embryo results were similar to the amphipod results. Significant reductions in copepod nauplii were observed in the Duwamish sediments. Microtox responded to the contaminated sediments but also showed "toxicity" in the Dosewallips reference sediments. The sea urchin fertilization assay showed effects in both the Duwamish and Dosewallips sediments. There may have been a "particulate effect" in this test.

Amphipods showed toxicity in organically amended sediments and showed elevated body burdens of organics. The larval surf smelt and oyster embryos were unaffected by organically-amended sediments. The oyster embryo survival relative to the seawater controls in amended and natural sediments = 51-72 %. Hence, there was elevated "mortality" in clean sediments relative to the seawater controls. Oyster embryo quality was suspect in some of the tests.

Nelson, P. O., C. K. Sollitt, K. J. Williamson and D. R. Hancock. 1984. Coos Bay offshore disposal site investigations. Interim Rpt., Phase II & III, April 1980 - June 1981 for Portland District, U. S. Army Corps of Engineers by Oregon State University, Corvallis, OR.

The authors used suspended and solid-phase sediment bioassays of Coos Bay sediments (4 stations) collected in 1980-1981 with a box corer. Sediments were stored at 4 °C until tested. Suspended phase = 1:4 ratio of sediment:Yaquina Bay seawater at 28‰ salinity, mixed and settled 1 hr; supernatant then used for the tests. For the solid-phase and bioaccumulation tests, used 2-cm layer of sediment on the bottom of aquaria. Algae growth was also tested with elutriates (= suspended phase + filtration at 0.45 µm).

Methods:

Suspended phase: Tested clams (*Macoma inclusa* and *Acila castrensis*), juvenile Dungeness crab (*Cancer magister*), sand dollar (*Dendraster excentricus*), and lugworm (*Abarenicola pacifica*). Used 1 to 20-liter aquaria, 28 ‰ salinity, 12 °C, 96-hr exposures with aeration, static system. Test endpoint = mortality. Five animals/aquaria with 3 reps/sediment.

Solid-phase: Tested clam (*Macoma inclusa*), sand shrimp (*Crangon* - mix of three species), sanddab (*Citharichthys sordidus*), lugworm, and amphipod (*Rhepoxynius epistomus*). Used 1 to 40-liter aquaria (glass or polyethylene) static system with aeration, 12 °C, 28 ‰ salinity, 10-day exposures, 2 reps/sediment, variable numbers of animals/aquaria, mortality endpoint.

Elutriate: Used 10-day growth test with marine algae (*Dunaliella tertiolecta*). Endpoint = standing crop biomass.

Bioaccumulation: Used 10-day exposures (with 24-hr post exposure purging) of lugworms to the solid phase.

Results:

Suspended phase: Dungeness crab survival = 86% at one station but this was not a significant reduction in survival over controls. Survivals for all other tests >90%.

Solid phase: No significant differences in mortalities over controls. However, *Crangon* and *Rhepoxynius* did suffer some mortalities >10%.

Elutriate test: Algae biomasses substantially less than for the controls but not significantly different due to high variability.

Bioaccumulation: Some bioaccumulation of metals was noted in *Macoma*.

Pierson, K. B., J. W. Nichols, G. C. McDowell and R. E. Nakatani. 1982a. Grays Harbor, Washington, dredged sediments: An assessment of potential chemical toxicity and bioaccumulation. Final Rpt., Contract Nos. DACW 67-80-C-0095 and DACW 67-82-C-0038, for the Seattle District, U. S. Army Corps of Engineers by Fish Res. Institute, University of Washington, Seattle, WA. 96 pp.

The authors used amphipod, crab zoea and chum salmon fry bioassays to test toxicity of sediment samples from three stations in Grays Harbor near Hoquiam, WA. Samples were collected with a stainless steel box corer and frozen prior to testing.

Methods:

Dungeness crab (*Cancer magister*) zoea: Used a 4-day elutriate test (1:4 ratio, stirred 30 min, settled 1 hr and filtered at 1.2 µm. Also ran 1:49 and 1:499 dilutions). Exposed 1st zoeal stage, 25 zoeae/chamber, 200 ml. seawater in glass beakers, 18 hr light:6 hr dark photoperiod. Several experiments looked at effects of filtration, feeding and interbucket (intersample) effects.

Chum salmon fry: 10-day exposures to the suspended-solids phase in a flow-through system. Fry exposed to 1.0, 0.5 and 0.25% solids with a diluter system. Used 50 salmon/15 gallon aquaria with 700 ml flow/min.

Amphipod (*Grandifoxus grandis*): Used 96-hr exposures to the solid phase in 2 liter beakers, 3 reps/sample, flow-through system with percolation of the seawater through the sediments, 75-100 ml/min flow of seawater.

Bioaccumulation: Exposed lugworms (*Abarenicola pacifica*), clams (*Macoma nasuta*) and juvenile sand sole (*Psettidichthys melanostictus*) to solid phase for 30 days, 10-30 animals/aquaria, 2 reps/sediment, 15 gal aquaria, flow-through system, 4-8 cm sediment, 250-400 ml/min flows of seawater.

Results:

Crab zoea: Seawater control mortalities = 8 and 12%. Test sediments caused up to 64% mortality in 96 hr. Filtration of the elutriates increased mortalities. No apparent effect of starvation on the test results.

Amphipods: Control sand survivals = 100%. Test sediments caused up to 13% mortality. Behavioral effects (increased swimming) were caused by the test sediments.

Chum salmon: No control or test mortalities observed and salmon grew during the 10-day exposures in all tanks.

Bioaccumulation: Up to 70% mortalities to *Macoma nasuta* in the 30-day exposures (possibly due to physical sediment factors). No accumulation of metals noted in any of the animals. Lugworms and sand sole did accumulate several organic chemicals (especially phthalates).

Pierson, K. B., B. D. Ross, C. L. Melby, S. D. Brewer and R. E. Nakatani.

1982b. Biological testing of solid phase and suspended phase dredged material from Commencement Bay, Tacoma, Washington. Final Rpt., Contract No. DACW 67-82-C-0038 for the Seattle District, U. S. Army Corps of Engineers by the Fish. Res. Institute, University of Washington, Seattle, WA. 59 pp.

Sediment testing was conducted on Commencement Bay (Blair and Sitzum Waterways) proposed dredged materials using a solid-phase amphipod assay, a suspended-solids phase salmon smolt assay and an elutriate assay with oyster embryos. Sediments were frozen prior to use. Supernatant from the thawed sediments was also tested with oyster embryos.

Methods:

Fall Chinook salmon smolts (*Oncorhynchus tshawytscha*): Exposed for 96 hrs in a flow-through system to the liquid-suspended phase at sediment concentrations of 0.25-1.0 % in 15-gal aquaria. Temperature range = 10.2-16.0 °C, salinity = 24.8-32.3 %, pH = 7.79-8.27, DO near saturation.

Oyster embryo (*Crassostrea gigas*): Exposed embryos for 48 hrs at 20.5 ± 0.5 °C in 800 ml of elutriates prepared by mixing 1:5 ratio of sediment:seawater for 30 min, settling 1 hr and filtering supernatant through 1.2 µm Whatman GF/C filters. Embryos were also exposed to water drained from the thawed sediment samples. Test pHs = 7.64-8.25.

Amphipods (*Grandifoxus grandis*): Exposed for 204 hrs in 2-liter beakers to the solid phase (4.5 cm depth) in a flow-through (percolating) system.

Results:

No salmon mortality or obvious adverse effects were observed due to sediment concentrations up to 1.0%.

No obvious effects due to the sediments noted in the amphipod assays, in part due to high control mortalities (6-17.5%) and high replicate variability. Some slight differences in burrowing in the test sediments were observed.

No significant effects in abnormal. for oyster embryos in the sediment elutriate tests. However, the control abnormalities were very high (up to 18.4% in a single replicate with the mean abnormal = 12.4%; n=9). Some toxic effects observed in the water decanted from the thawed sediments, but 100% abnormal was also found for decanted water from Wollochet Bay (reference) sediments. Embryo mortality was not assessed--no initial counts were taken in the seawater controls.

PTI Environmental Services. 1988. Puget Sound Dredged Disposal Analysis Baseline Study of Phase I disposal sites. Final Rpt., PTI Contract C721-07, for the Washington Department of Ecology, Olympia, WA by PTI Environmental Services, Bellevue, WA. 126 pp. + appendices.

This study used amphipod, mussel embryo and Microtox bioassays to measure toxicity of sediments collected from Commencement Bay, Elliott Bay and Port Gardner PSDDA Phase I disposal sites (and nearby benchmark stations). Bioassay samples were collected between May 17-24, 1988. Concurrent measures of sediment chemical concentrations and benthic infauna also made. REMOTS profiles also taken at each station.

Methods:

All bioassays were conducted by EVS Consultants, Vancouver, B. C. Test sediments were composited from 6 van Veen grabs at each station and split for chemistry and bioassays. Storage temperature was not specified but was probably 4 °C. Generally used PSEP protocols for the bioassays but the specific test conditions (e.g., amounts of sediment used, aeration, settling times, etc.) were not spelled out in the report.

Results:

Amphipod (*Rhepoxynius abronius*) 10-day mortality: West Beach control sediment mortality = 6%. 96-hr LC50 for NaPCP (reference toxicant) = 240 µg/liter. Salinity = 29-32‰ and exceeded PSEP-specified range of 27-30‰ in 79 of 85 cases. Temperature = 14-15 °C, DO = 6.0-8.2 mg/liter and pH = 7.5-8.0. Carr Inlet reference sediment mortality = 16%, Port Susan reference mortality = 37%. There were significant mortalities in 3 Commencement Bay

sediments (with high standard deviations) and in 3 Port Gardner test sediments. Fine grain size may have affected some of the responses.

Mussel (*Mytilus edulis*) 48-hr larval test: Mean mortality in the West Beach control sand = 62.8% and mean abnormal = 9.3%. Thus, this test failed PSEP QA/QC guidelines for mortality ($\leq 30\%$). No seawater control mortality/abnormality given. The 48-hr LC50 for NaPCP was $<100 \mu\text{g/liter}$. Salinity = 29-31 ‰ (exceeding PSEP-specified range in 84/85 cases). Temperature = 17-19 °C and was below specified PSEP range in 80 of 85 cases. DO = 5.4-6.9 mg/liter, pH 7.3-7.7. The test data were not discussed in the report but the raw data appear in the appendices.

Microtox 15-min EC50: No dose-response to West Beach control sediment. 15-min EC50 for sodium arsenate (reference toxicant) = 7.6 mg/liter. No significant toxicity noted in any of the test sediments.

Tetra Tech, Inc. 1985. Commencement Bay nearshore/tideflats remedial investigation.
Volume 1. Final Rpt. TC-3752 for the Washington Department of Ecology and the U. S. Environmental Protection Agency by Tetra Tech, Inc., Bellevue, WA.

This study used amphipod and oyster embryo bioassays and bioaccumulation tests to measure toxicity of sediments from Commencement Bay. Sediments were collected in March and July 1984 from 52 stations. Also, tested sediments from four stations in Carr Inlet (= reference sediments). Samples were collected by van Veen grab.

Methods:

Amphipod (*Rhepoxynius abronius*): Used the 10-day "Swartz test" with unfrozen sediments. Used 2 cm sediment depth in 1-liter glass jars with 800 ml seawater at 28 ‰ salinity, 20 amphipods/jar, 5 reps/sample, $15 \pm 1^\circ\text{C}$ with constant light. Used CdCl_2 as a reference toxicant.

Oyster embryo (*Crassostrea gigas*): Used a 48-hr test at $20 \pm 1^\circ\text{C}$ with 15 g sediment (wet weight) per chamber with a total volume of sediment + seawater of 750 ml contained in plastic bottles, 2 reps/sample. Sediments were mixed by shaking for 5 sec prior to embryo addition. Larvae were concentrated at termination with 38-µm mesh Nytex screen. Salinity, DO, and pH levels adjusted to 28 ‰, 7.6-7.8 mg/liter and 7.9-8.0, respectively.

Bioaccumulation: Exposed English sole (*Parophrys vetulus*) and Dungeness crab (*Cancer magister*) to solid phase sediments. The English sole were also examined for histopathological effects.

Results:

Amphipods: Control mortality = 4 to 10%. One Carr Inlet reference sample caused an average mortality of 85% (this sample was deleted from subsequent statistical comparisons). Average mortalities in 18 test samples were significantly different from the reference samples. Some sediments were still toxic with dilutions up to 50-75% and one was still toxic at a 10% concentration (=90% dilution).

Oyster embryo: No initial counts taken in the seawater control jars for subsequent control mortality (thus, 48-hr control survival set to 100%). Mean control abnormal = 4%. 15 test samples showed significantly increased abnormalities. Mortalities in the test sediments generally agreed with the trends in abnormalities. Relative sediment control mortality = 30.1% and abnormal = 8.1%.

Bioaccumulation: Copper was significantly elevated in English sole muscle tissue. Lead and mercury were significantly elevated in Dungeness crab. PCBs were detected in all sole and were highest in samples from the Commencement Bay waterways. DDE was also detected at low concentrations in samples from all areas.

Ward, J. A., J. Q. Word and L. D. Antrim. 1989. Biological testing of sediment for the Olympia Harbor Navigation Improvement Project, 1988. Geoduck, amphipod and echinoderm bioassays. Final Report for the U. S. Army Corps of Engineers, Seattle District by Battelle Pacific Northwest Laboratory, Sequim, WA. PNL-6883/UC-11.

This project evaluated the acceptability of the Olympia Harbor Navigation Improvement Project sediments for open-water disposal in Puget Sound under the new PSDDA guidelines. This portion of the work used biological testing (geoduck, amphipod, oyster embryo and sea urchin embryo) to assess the acceptability for disposal. Microtox testing was conducted for this project by NMFS/NOAA, Seattle. Chemical testing was also conducted by NMFS. All samples were stored at 4 °C for <6 weeks.

Methods:

Geoduck, *Panopea generosa*: Conducted "protocol development" tests first and then "definitive tests" with the Olympia Harbor sediments. For "protocol" tests, sediment depths of 1.5, 2.0 and 5.0 cm of fine-grained sediment from Sequim Bay and coarse-grained sediment from Point Whitney were tested. These were 10-day exposures in a flow-through system at 40 liters/min, equilibration for 2 hrs at the start of the test, 1 liter jars, temperature = 15 ± 1 °C, salinity = 31 ± 1 ‰, pH = 8.0 ± 0.5 , DO ≥ 6.0 mg/liter and mortality as an endpoint. Geoducks = 8-10 mm in length. Used 2 cm of sediment for the "definitive test" with other conditions as above.

Amphipod, *Rhepoxynius abronius*: Used the "Swartz protocol" with 3 cm (150 ml) sediment in 1-liter glass jars, 800 ml seawater at 15 °C, 10-day exposures, static system and mortality as the test endpoint. NaPCP and CdCl₂ were used as positive controls.

Sea urchin (*Strongylocentrotus purpuratus*) embryo and sperm assays: For the embryo assays, used 15 g sediment/750 ml total seawater volume, 4-hr equilibration, static system, 12 °C, 72-hr exposures, mortality and abnormality test endpoints, ~30 embryos/ml starting density, beakers decanted at the end and no sediment checks for entrained larvae. Initial fertilization success for Test #1 = 77% and for Test #2 = 88%. For the sperm assays, used the protocol of Dinnel et al. (1987). 10-ml volumes tested from the embryo beakers, 60-min sperm exposure times, 20-min sperm/egg contact times, 12 °C, and a 200:1 sperm:egg ratio.

Oyster larvae (*Crassostrea gigas*) embryo assay: Used PSEP protocol. 15 g sediment with 750 ml total volume, 48-hr exposures at 20 °C. The oysters were in poor spawning condition; thus, they were "strip spawned."

Results:

Oyster embryo test: All tests failed control survival criteria of $\geq 70\%$. Thus, switched to the sea urchin embryo assay.

Sea urchin embryo test: Mean abnormal in all test and control sediments = 0-5%. The seawater control survival = 90%. Ranges for the test sediment survivals = 19-86%. West Beach sediment = 70-& 86% survivals. Test sediments = 19-46% survivals. Sequim Bay reference sediment = 19 & 31 % survivals. Seven of 13 Olympia test sediments showed significant differences from West Beach Control sediment, but none were different from the Sequim Bay reference sediment survivals.

Sperm assay: Mean fertilization success in all control and test sediments $\geq 93\%$ (no significant differences).

Geoduck assay: Geoducks had trouble digging into coarse sand. Survivals were highest in the 1.5 and 2.0 cm treatments. Lowest survivals were in Olympia Harbor and West Beach sediments (58%). Range of other survivals = 59-85%. Sequim Bay reference sediment = 70% survival. This test needs more development before routine use and better availability of proper size (<5 mm) animals.

Amphipod assay: 10-day survivals ranged from 0% (Sequim Bay reference) to 98% for West Beach control sand (although a second Sequim Bay sample showed 92% survival). Survival range for Olympia Harbor sediments = 66-87%. No test sediments were statistically different from Sequim Bay reference (92%), but four were different from the West Beach controls. LC50 for 4-day NaPCP toxic control = 0.26 mg/liter.

Westley, R. E., T. Schink, A. J. Scholz, C. L. Goodwin, R. Gerke and M. Tarr.
1972. A preliminary evaluation of the toxicity of the bottom sediments of Olympia Harbor.
Final Rpt. by Washington Department of Fisheries, Olympia, WA. 51 pp. + appendix.

Sediments from Olympia Harbor (collected Feb. 1972) were tested for toxicity using Pacific oyster (*Crassostrea gigas*) embryo, phytoplankton (*Skeletonema* sp.) and pink salmon fry bioassays. Concentrations of chemical contaminants were also measured.

Methods:

Oyster embryo tests: Evaluated various exposure types and times in preliminary tests with one Olympia Harbor sediment. Generally used "Woelke's Protocol" without the termination filtration step. Used 4-6-hr and 48-hr exposures, $20 \pm 0.5^{\circ}\text{C}$, salinity $\geq 20\text{‰}$ and sediments loads of 0.2-4.0 g/liter (dry weight). Olympia Harbor and Oro Bay (control) sediments were assayed at concentrations ranging from 0.2 to 11.1 mg/liter (dry).

Phytoplankton tests: Used 1, 24 & 48-hr exposures to sediment concentrations of 0.12 to 3.0 g/liter (dry) with culture medium in the samples. Test endpoints = photosynthetic rate, number of cells at termination, and amount of cell settling.

Pink salmon fry: Used 38-mm fish acclimated to seawater for 5 days prior to assays. Assays were conducted in 30 liters of seawater in concrete tanks. Used 1% and 5% sediment

slurries (by wt.), 48-hr exposures with 24-hr observations for delayed mortalities following termination of the exposures.

Results:

Oyster embryos: The 4-6-hr abbreviated assay (using almost developed larvae) was slightly less sensitive to sulfite waste liquors than the 48-hr assay. Filtration of samples at termination underestimated % abnormalities in the samples. Small amounts of added sediments did not affect the toxicity of sulfite waste liquors. The method of bioassay had major affects: Most toxic = machine or hand-agitated sediments followed by sediment mixed once = supernatant from 15-min settling. Least toxic = sediment in chamber but unmixed. Olympia Harbor sediments varied in toxicity (used hand-agitation method). The control sediments from Oro Bay were least toxic. All pHs stayed between 7.6-7.9.

Phytoplankton: The test sediments generally stimulated photosynthetic rates and the number of cells. However, the sediments also caused cells to settle in the cultures.

Pink salmon fry: No significant mortalities in the test sediment slurries at either 1% or 5%. Sediments did cause some initial disorientation and modifications of swimming behavior.

Williams, L. G., P. M. Chapman and T. C. Ginn. 1986. A comparative evaluation of marine sediment toxicity using bacterial luminescence, oyster embryo and amphipod sediment bioassays. *Marine Environ. Res.* 19:225-249.

The authors used Microtox (saline), amphipod (*Rhepoxynius abronius*) and oyster embryo (*Crassostrea gigas*) assays of sediments from 46 stations in Commencement Bay and 4 reference stations in Carr Inlet.

Methods:

Amphipod: Tested 2 cm sediment in 1-liter glass jars, 10-day exposures in an aerated static system (Swartz method), with mortality as the test endpoint.

Oyster embryo: Used Chapman and Morgan method with 15 g sediment + 750 ml seawater in 1-liter sealed polyethylene bottles, 48-hr exposures at 20 °C. Test endpoint = live, normal shelled veligers (thus combining mortality and abnormality into one value??).

Microtox: Tested saline extracts, unspecified volume of sediment (13.0-26.4 g) washed for 24 hrs in 10 ml Microtox dilutent (2% NaCl in DD H₂O) in the dark at 4 °C with agitation. The supernatants were diluted to 0, 12.5, 25, 50 and 100% dilutions and both 5 and 15-min exposure test times were used. Sodium arsenite was the reference toxicant.

Results:

Mean amphipod mortality in the Commencement Bay sediments = 6-100% and significantly elevated mortality above the Carr Inlet reference sediments was observed in 17 stations (39%).

Oyster embryo mean seawater control abnormal = 4.1%. Mean Carr Inlet reference sediment abnormal = 13.1%. The difference between seawater control and the reference sediment abnormalities was attributed to physical loss (attachment of normal larvae to the sediments) of larvae in the reference sediments. Mean abnormal for the Commencement Bay sediments ranged from 8.5-100% and was significantly higher than Carr Inlet sediments in 16 samples (35%).

Microtox tests showed no significant differences between the 5 and 15-min exposures. Salinity had only a small effect ($\pm 3\%$) over the range tested (18-24%). No change in response observed over the 30-day sediment storage period. For Commencement Bay sediments, decreased luminescence = -4.6 to 94.6% and 29 sediments caused significant decreases (63%) over Carr Inlet sediments (note: minus values = stimulation of light emission).

The authors established a sediment toxicity index by adding together the individual test ranks. Kendall's coefficient of concordance for the 3 bioassays = 0.64, $p < 0.001$. The Pearson correlation coefficients were:

Oyster/amphipod	$r = 0.86$	
Oyster/Microtox	$r = 0.62$	(all significant at $p < 0.001$)
Amphipod/Microtox	$r = 0.48$	

41% of the Commencement Bay sediments were toxic in all 3 tests, and 41% not toxic in all 3 tests.

Word, J. Q., J. A. Ward, C. W. Apts, D. L. Woodruff, M. E. Barrows, V. I. Cullinan, J. L. Hyland and J. F. Campbell. 1988. Confirmatory sediment analyses and solid and suspended particulate phase bioassays on sediment from Oakland Inner Harbor, San Francisco, California. Final Rpt. PNL-6794/UC-11 for the U. S. Army Corps of Engineers, San Francisco District by Battelle Pacific Northwest Laboratory, Sequim, WA.

The authors conducted physical/chemical/biological testing of sediments from Oakland Harbor, California. Biological testing included solid phase assays with polychaetes, clams and 2 species of amphipods; suspended phase assays with mysids, juvenile flatfish and oyster embryos; and bioaccumulation tests with the clam *Macoma nasuta*. Sediments were collected in late March 1988, stored at 4 °C, and tested in April. The bioassays generally followed the specifications of the COE/EPA (1977) "Implementation Manual."

Sediment preparation:

Suspended-particulate phase (SPP): The sediments were mixed 1:4 with seawater for 30 min, settled for 10 min and centrifuged for 10 min to provide the test supernatant (note: this departure from the COE/EPA procedure was necessary due to the high level of material remaining in suspension after the usual 1-hr settling time—J. Q. Word, pers. comm.).

Solid phase (SP): Some water was used to sieve the sediments. 3 cm reference sediment + 1.5 cm overlayer of test sediment used in test containers. Reference sediments = 4.5 layer. Two reference sediments were collected off Pt. Reyes and from mid Sequim Bay.

Biological SPP test methods:

Mysids (*Acanthomysis sculpta*): Used 96-hr static exposures with 6 sediments at 0, 10, 50, and 100% SPP, 3 replicates for each sediment with 10 mysids/rep in 1-liter beakers with no aeration, mortality endpoint. Fed shrimp nauplii 2X daily. Temp. = 15 ± 1 °C, salinity 31 %o, DO > 4 mg/liter.

Juvenile speckled sanddab (*Citharichthys stigmaeus*): Used 96-hr static exposures with aeration with 6 test samples, 3 reps/sediment with 10 dabs/rep in 20 liters seawater, no food, with SPP concentrations of 0, 10, 50 and 100%. Mortality endpoint with some histopathology assessments of the livers. Temp. = 15 ± 1 °C, salinity 31 %o, DO > 4 mg/liter.

Pacific oyster embryos (*Crassostrea gigas*): Used a 48-hr static test with aeration at SPP concentrations of 0, 10, 50 and 100% in 800 ml seawater in glass Mason jars, 6 sediment samples with 3 reps/sediment. Mortality and abnormality endpoints. Temp. = 20 ± 1 °C, salinity 25 ± 2 %o.

Biological SP test methods:

Polychaete (*Nephtys caecoides*): Used 10-day flow-through exposures with 20 sediments, 3 reps/sediment with 20 worms/rep in 6 liters sediment and 30 liters seawater. Temp. = ??, salinity = ?? Mortality endpoint.

Clam (*Macoma nasuta*): Same conditions as for the polychaete tests. This species was also used for the bioaccumulation tests following a 48-hr depuration period.

Amphipod (*Rhepoxynius abronius* and *Grandidierella japonica*): Used static, 10-day exposures to 225 ml sediments with 575 ml seawater with aeration. Sediments were added to beakers and allowed to settle overnight and 75% of water replaced prior to addition of amphipods. 5 replicates of 20 test sediments with 20 *Rhepoxynius* and 10 *Grandidierella*/rep. Temp. = 15 °C, salinity = ?? Mortality endpoint.

Results:

Polychaete, clam and *Grandidierella* assays with SP showed no significant mortalities in the test sediments. *Grandidierella* showed poor survival in the reference sediments which invalidated this test. *Rhepoxynius* showed significant mortality in 4 of 20 test sediments. For the SPP tests, the mysids showed significant mortality in 5 of 6 test sediments, but not enough mortality in 100% SPP to calculate EC50s. For the dabs, significant mortality was observed in 1 of 6 sediments, but calculations of EC50s also were not possible. For oyster embryos, 3 of 6 sediments caused significant abnormality compared with the seawater controls. Also, 3 of 6 sediments showed elevated mortality (same samples as for abnormality). There were no obvious correlations between the biological responses and the chemical contaminant concentrations. For bioaccumulation, *Macoma* showed significant burdens of lead and chromium from some sediments as compared to the Pt. Reyes reference sediment. PAHs were not accumulated and organotin showed elevated tissue concentrations from all sediments.

Word, J. Q., J. A. Ward and A. L. Squires. 1989. The results of chemical, toxicological, and bioaccumulation evaluations of dioxins, furans, and guaiacol/organic acids in sediments from Grays Harbor/Chehalis River area. Final Report for Seattle District, U. S. Army Corps of Engineers by Battelle Marine Sciences Laboratory, Sequim, WA.

This study evaluated the toxicity of Grays Harbor sediments using amphipod (*Rhepoxynius abronius*) and clam (*Macoma nasuta*) survival; elutriate toxicity to Pacific oyster (*Crassostrea gigas*) larvae; and light diminution in the Microtox assay. Bioaccumulation of dioxins/furans was also evaluated using 30 and 60-day exposures of *Macoma* to sediments. Field-collected adult Dungeness crab (*Cancer magister*) were also measured for tissue (muscle and hepatopancreas) concentrations of dioxins and furans.

Methods:

Test sediments were collected in Grays Harbor from 31 July to 5 August 1989 and stored at 4 °C. Sampling devices = 0.1 m² van Veen grab, a vibracore and a dart core. Most sediment samples were composites of multiple grabs/cores. Control sediments were from West Beach, Whidbey Island and Sequim Bay.

The elutriates were prepared by adding sediment to water in a 1:4 ratio in 0.45 µm-filtered seawater, shaking 30 min, settling 10 min and centrifuging 10 min at 1,750 RPM.

Microtox: Used saline extract protocol (PESP 1986) with 30 g of sediment. Test endpoint = 15 min EC50 for light reduction.

Amphipod: Used the Swartz et al. (1985 = PSEP) protocol. Used 2 cm sediment in 1 liter beakers, 5 reps/station, 10 day exposure at 15 °C, 20 amphipods/beaker, amphipods collected from West Beach, and test endpoint = death.

Oyster larvae: Oysters were conditioned to spawn for 4-6 weeks at 20 °C with feeding. Test protocol = Suspended Phase Particulate Method of ASTM Method E724-80 (ASTM 1980) [Note: the Materials and Methods section of the report indicates that the "elutriate" (prepared as indicated above) was used—not a "suspended phase particulate" preparation]. Tests were conducted at 20 °C for 96 hrs (??—most oyster larvae tests at 20 °C are 48 hr exposures). Stocking density = 15-30 fertilized eggs/beaker and initial stocking density was assessed in 10 replicate counts. Test endpoint = survival and development to a normal "D"-shaped veliger.

Clam: *Macoma* were collected from Discovery Bay and exposed to sediments in 38 liter aquaria at 15 °C for 30 or 60 days. Test endpoints = survival and bioaccumulation of dioxins and furans.

Results:

Microtox: The EC50s for the reference toxicant (sodium arsenate) = 7.7 - 18.9 mg/liter (as arsenic). Most test sediments produced light enhancement. One station produced a light decrease. No EC50s could be calculated due to insufficient responses.

Amphipod: Control survivals = 87% (Sequim Bay) and 96% (West Beach). None of the survivals in the test sediments were significantly reduced (range = 75-95% average survival).

Oyster larvae: Mean survival to "D"-shaped veliger was 92% in West Beach and 88% in Sequim Bay sediments. Only 2 of 17 test sediments caused a significant reduction in survival to normal veliger (49% and 53%).

Clam: There were no significant reductions in *Macoma* survivals in either 30 or 60- day exposures. Small amounts of dioxins and furans were accumulated in the tissues.

WATER COLUMN

Dinnel, P. A., Q. J. Stober, J. M. Link, M. W. Letourneau, W. E. Roberts, S. P. Felton and R. E. Nakatani. 1983. Methodology and validation of a sperm cell toxicity test for testing toxic substances in marine waters. Final Report for Washington Sea Grant and the U. S. Environmental Protection Agency by the Fish. Res. Institute, University of Washington, Seattle, WA. FRI-UW-8306:208 pp.

This is essentially a University of Washington numbered report version of Dinnel's Ph.D. Dissertation (Dinnel 1984—see this entry below for details of this work).

Dinnel, P. A. 1984. Methodology and validation of a sperm cell toxicity test for testing toxic substances in marine waters. Ph.D. Dissertation, School of Fisheries, University of Washington, Seattle.

This work primarily investigated the use, refinement and comparative sensitivity of a sea urchin sperm cell bioassay for marine pollution monitoring. This test exposed sea urchin or sand dollar sperm cells to test solutions for 60 min prior to fertilization of the eggs. Elevation of the fertilization membrane was used as the test endpoint. An oyster sperm and a salmon sperm test (for brackish waters) were also investigated.

The "validation" portion of this work compared the sensitivity of sperm cell tests to other common test organisms available in the Pacific Northwest. Toxicants tested were Cu, Cd, Pb, Ag, Zn, DDT, Dieldrin, Endrin and Endosulfan. The chemical interactions, degradation and solubility of these toxicants in seawater were also investigated and a literature review for these toxicants presented.

Methods:

The following toxicant tests and conditions for those tests were as follows (all toxicant concentrations were measured by AAS or GC):

Organism	Test Type*	Exposure Time (hr)	Temp. °C	Salinity ‰	pH	DO (mg/liter)
Sea urchin sperm	S	1	12.0	30	~8	-----
Sea urchin embryos	S	120	8.3	30	~8	-----
Sand dollar embryos	S	72	12.5	30	~8	-----
Oyster embryos	S	48	20.0	30	~8	-----
Mussel embryos	S	72	12.5	30	~8	-----
Crab zoea	S	96	8.5	30	~8	-----
Larval squid	S	96	8.6	30	~8	-----

Organism	Test Type*	Exposure Time (hr)	Temp. °C	Salinity ‰	pH	DO (mg/liter)
Larval cabezon	S	96	8.3	27	~8	-----
Sand shrimp	F-T	96	10-14	28-30	~8	>5
Shiner perch	F-T	96	13-14	29-30	~8	>5
Coho salmon smolts	F-T	96	11-13	28-29	~8	>5
Sea urchin adult	F-T	96	9-11	29	~8	>5
Sand dollar adult	F-T	96	12.3	29	~8	>5
Mussel adult	F-T	96	12.0	29	~8	>5

* S = Static test; F-T = Flow-through test

Results (EC50s or LC50s in µg/liter — except lead & cadmium = mg/liter):

Organism	Cadmium	Copper	Lead	Silver	Zinc
Sea urchin sperm	12-16	2-59	1-19	85-115	148-313
Sand dollar sperm	8	26	13	55	28
Oyster sperm	12	12	5.5	29	444
Salmon sperm	1.5	44	33	11	1,208
Sea urchin embryo	0.5-2	6-21	<9.7	15-24	23-50
Sand dollar embryo	7.4	33	<1.5	33	<820
Oyster embryo	<1.1	6	0.7	19	206

Organism	Cadmium	Copper	Lead	Silver	Zinc
Mussel embryo	<6.5	<35	>9.5	<4.4	<314
Crab zoea	0.25	96	0.6	33	586
Squid juvenile	>10	309	>2.1	100-200	>1,920
Cabezon juvenile	<0.5	95	1.5	>800	191
Coho salmon smolt	1.5	601	NT*	488	NT
Sand shrimp	2.3	898	>2.1	>838	NT
Mussel adult	3.4	NT	NT	NT	NT
Green urchin adult	>0.7	NT	NT	NT	NT
Sand dollar adult	>4.0	NT	NT	NT	NT
English sole juvenile	NT	NT	NT	800	NT

NT = Not tested

Pesticide LC50s or EC50s, all in µg/liter:

Organism	DDT	Dieldrin	Endosulfan	Endrin
Sea urchin sperm	1-3	1.4->92	81-780	103-342
Sand dollar sperm	NC	88	352	441
Oyster sperm	0.4	52	215	124

Organism	DDT	Dieldrin	Endosulfan	Endrin
Salmon sperm	>2.4	124	>765	>345
Sea urchin embryo	>8.2	143	227->549	221->359
Sand dollar embryo	>17.2	>68	822	>362
Oyster embryo	>4.6	23	55	152
Mussel embryo	>17.2	48	212	>362
Crab zoea	1.1	24	15	2.0
Coho salmon smolt	NT	NT	1.7-2.5	1.2
Sand shrimp	NT	NT	2.3-4.1	0.45
Shiner perch	NT	NT	1.1	0.5
Mussel adult	NT	NT	NT	>2.2
Sand dollar adult	NT	NT	NT	>2.2

NC = Not calculated

NT = Not tested

Dinnel, P. A., J. M. Link, Q. J. Stober, M. W. Letourneau and W. E. Roberts.
 1989. Comparative sensitivity of sea urchin sperm bioassays to metals and pesticides.
 Arch. Environ. Contam. Toxicol. 18:748-755.

The objective of this work was to compare the sensitivity of a standardized sea urchin sperm/fertilization assay to the responses of embryo, larval and adult marine organisms to five metals (Ag, Cd, Cu, Pb, Zn) and four pesticides (DDT, Dieldrin, Endrin, Endosulfan) in natural seawater.

Methods:

Sperm/fertilization assays were conducted with gametes from three species of sea urchin (*Strongylocentrotus droebachiensis*, *S. purpuratus* and *S. franciscanus*) and one sand dollar (*Dendraster excentricus*) and the results compared to assays using urchin embryonic development success; Dungeness crab, squid and cabezon larvae or juveniles; and "adult" animals (Coho salmon smolts, sand shrimp and shiner perch). This article is the publication version of the contents of Dinnel's Ph.D. Dissertation (Dinnel 1984—see above).

The sperm assays used 60-min static pre-exposures of the sperm followed by 20-min sperm/egg interaction times. Successful elevation of the egg fertilization membrane was the endpoint. Urchin embryo assays used 72-120-hour static exposures of the developing embryos with abnormality being the test endpoint. Larval tests used 96-hour static exposures in 250 ml beakers with mortality as the test endpoint. The adult animal exposures were 96 hours in a flow-through test system. See Dinnel (1984) above for additional details of the test conditions.

Results:

Average EC50s or LC50s were calculated for most of the tests or ranges of toxicity given where calculations were not possible. Sperm assay results tended to group with the embryo assay results while the larval responses tended to group with the adult animal responses. Sperm and embryo assays were most sensitive to the metals while the larval and adult assays were most sensitive to the pesticides. See Dinnel (1984) above for specific toxicity values.

In conclusion, a sperm assay is a quick, sensitive (to many toxicants), easy, economical and universal (world-wide) bioassay system for measuring toxicity in marine waters.

Nacci, D., E. Jackim and R. Walsh. 1986. Comparative evaluation of three rapid marine toxicity tests: Sea urchin early embryo growth test, sea urchin sperm cell toxicity test and Microtox. *Environ. Toxicol. Chem.* 5:521-525.

The authors used three rapid (<5 hr exposure times) bioassays to assess toxicity of 8 organic chemicals and 5 metals.

Methods:

- 1) Early sea urchin, *Arbacia punctulata*, embryo growth (4-hr) test used 2 hr growth in toxicant solutions followed by addition of ^3H thymidine and incubation for 2 more hours. Test endpoint was the degree of ^3H thymidine incorporation into the growing embryo.
- 2) Sea urchin sperm/fertilization assay with *Arbacia* using the "Dinnel Protocol."
- 3) Microtox 15-min EC50 at 15 °C. Both organic and saline extracts used.

Results:

For organics: Toxicity rankings for 6/8 of the toxicants were the same for the embryo growth vs. the sperm tests. 7/8 rankings were the same for the embryo growth vs. Microtox. Early embryo growth and Microtox results were significantly correlated ($r^2 \geq 0.88$) with two other acute (fish and *Daphnia*) LC50s.

For metals: The toxicity rankings were similar between the rapid tests but not between the rapid and acute tests. There was a high correlation ($r^2 = \geq 0.81$) between the results of East and West coast embryo and sperm assays.

SCCWRP. 1989. Comparative wastewater toxicity tests. Pp. 72-77 *In: South. Calif. Coast. Water Res. Proj. Annual Report 1988-1989*, Long Beach, CA.

This study investigated the relative toxicity of seven southern California sewage treatment plant effluents using sea urchin sperm and embryo assays and the Microtox test. It also used the sperm assay to determine loss in toxicity of effluents held up to 48 hrs.

Methods:

Sea urchin (*Strongylocentrotus purpuratus*) sperm/fertilization assays were conducted following the methodology of Dinnel et al. (1987): 60-min sperm exposures to 0.01-4% effluent (no other test conditions given). Sea urchin embryo assays were 48-hr exposures of the developing embryos with test endpoints of abnormal development and echinochrome pigment production. The Microtox test used 30-min exposures of *Photobacterium phosphoreum* (probably in a 2% NaCl matrix at 15 °C) with light decrease as the test endpoint.

Results:

Sperm/fertilization assays were the most sensitive indicators of effluent toxicities. No observable effects concentrations (NOECs) ranged from 0.1 to >2% for the effluents with the JWPCP effluent being the most toxic. Microtox was next in sensitivity and the embryo test least sensitive.

This testing round showed reduced toxicity from all treatment plants as compared to past testing and correlated with improved treatment and generally reduced toxicant loadings. Repetitive sperm tests conducted over 48 hrs with the same effluent samples generally showed reduced toxicity through time, possibly due to the loss of volatile toxicants, degradation, or adsorption to the test containers.

REVIEWS AND MISCELLANEOUS

Benedict, A. B. and E. R. Long. 1987. A catalog of biological effects measurements along the Pacific Coast. NOAA Tech. Memo. NOS OMA 32. 11 pp. + appendices.

The authors compiled a listing of 170 studies associated with biological effects of pollution on the West Coast of the U. S., Canada and Hawaii. 305 different types of tests are identified with 107 species and 16 communities used as biological indicators. This report spans the period from 1951 to 1987 and covers water and sediment bioassays, cytotoxicity/genotoxicity tests with fish cells, various sublethal bioassays, fish histopathology and benthic infaunal sampling. The greatest amount of data come from Puget Sound followed by the Southern California Bight.

Types of information cataloged include: Study ID, project title, duration, sampling frequency, location, number of stations, investigator/affiliation, types of tests, endpoints, taxon, authors, and publication/report citations.

Olsen, L. A. 1984. Effects of contaminated sediment on fish and wildlife: Review and annotated bibliography. Final Rpt. for the U. S. Fish and Wildlife Service, Washington, D.C. FWS/OBS-82/66. 103 pp.

This review provides brief overviews of availability, bioaccumulation and "summary" of the effects of sediments contaminated with heavy metals, petroleum hydrocarbons, synthetic organic compounds and radionuclides. It also provides an annotated bibliography of several hundred sediment-related studies/publications.

Tetra Tech, Inc. 1985a. Everett Harbor Action Plan: Initial data summaries and problem identification. Final Rpt. TC-3991-03 for the U. S. Environmental Protection Agency, Region X, Seattle, WA by Tetra Tech, Inc., Bellevue, WA. 81 pp. + appendices.

This report gives general overviews and data summaries for water column, sediment and bioaccumulation testing conducted in Everett Harbor prior to 1985. Testing discussed includes salmonid, oyster embryo, amphipod, oligochaete respiration bioassays and genotoxicity/mutagenicity tests with fish cell cultures. Some of these tests used frozen sediments. Also, it includes information on bioaccumulation in English sole tissues.

This report is a review only—No original data are presented.